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NITRATES, NITRIFICATION, AND BACTERIAL CONTENTS OF FIVE TYPICAL ACID SOILS AS AFFECTED BY LIME, FERTILIZER, CROPS, AND MOISTURE

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INTRODUCTION

The decay of organic matter and the transformation of nitrogen from one chemical combination to another were known and studied long before bacteria were isolated. These phenomena were attributed to purely chemical agencies until the discovery of the function of soil bacteria proved them to be almost entirely due to microorganic life. Most investigations in soil bacteriology have dealt with either the products of bacterial activities without reference to the number of organisms present or with only an enumeration of the bacteria present in the soil. This paper presents the results of an investigation taking into consideration both nitrates and bacterial numbers, as well as a correlation of the two, under certain specific conditions.

HISTORICAL REVIEW

NITRIFICATION

The difficulties attendant upon keeping an adequate supply of available nitrogen in the soil are so great that those bacterial activities which have to do with nitrate formation are important and have been extensively studied. As early as 1660 Digby (*2*)[1] mentioned the value of nitrates in agriculture. He attributed the growth of plants to the "nutritional and attractional" powers of a "nitrous salt." Many agricultural writers of the early part of the nineteenth century followed the lead of Liebig, who claimed that nitrogen was not needed as a soil amendment. In 1856 Boussingault and Ville (*8*) independently published experimental results which proved that nitrates are markedly beneficial to plant growth, but it was not until 21 years later that Schloesing and Müntz (*22*) demonstrated that nitrification in the soil was due to organized ferments and does not take place in the absence of these ferments.

[1] Reference is made by number (italic) to "Literature cited, p. 41-42."

Nitrification in soils is dependent upon several different factors, and chemists have not entirely agreed as to the conditions necessary for it to take place. It was early observed that calcareous material was necessary for the preparation of niter beds. Thouvenel (*4*) in 1787 found chalk and carbonate of lime to favor nitrification more than a number of earths and other chemicals. From the accumulated evidence that carbonate of lime increased nitrate formation and the fact that acid forest soils often contained no nitrates the conclusion was reached by many investigators that nitrification did not take place in an acid soil. In 1891 Warington (*25, p. 51*) said:

> A further condition of nitrification is the presence of a base with which the nitric acid when formed may combine. This condition is quite essential. Nitrification can only take place in a feebly alkaline medium.

A little later in 1894 Dehérain (*8*) (*p. 360*) made the following statements:

> The nitric ferment does not act in an acid medium . . . . . It is true that nitrification may go on in soil deficient in lime . . . . . Moreover, the application of carbonate of lime to such soils is very beneficial and increases the production of nitrates.

NITRATES IN ACID SOILS

Twenty-two years before Warington (*26*) stated that nitrification could only "take place in a feebly alkaline medium" Houzeau (*12*), in 1872, reported nitrification in an acid soil. In 1908 Hall, Miller, and Gimingham (*11*) found nitrates in an acid soil, but believing that nitrification could not take place in an acid medium, they attributed the phenomena to the probable presence in the soil of small isolated particles of calcium carbonate. Since 1908 several workers have reported nitrification in acid soils. In 1913 Petit (*21*) found pronounced evidence of such a condition, while the same year Abbott, Conner, and Smalley (*1*) reported the presence of large amounts of nitrates in an excessively acid soil. The water extract of the soil was acid in reaction and contained considerable aluminium. The next year Temple (*23*) reported nitrification in acid or nonbasic soils. White (*26*) in 1915 from investigations on some unlimed and limed plots at the Pennsylvania Station found that nitrification was very active in many very acid areas. White remarks that—

> These results are entirely contrary to the general belief that nitrification ceases on very acid soils.

Since nitrification is the result of oxidation reactions and due to bacteria, it is affected by soil moisture and aeration. Schlösing (*8*), in 1868, found that rapid loss of nitrates occurred when a moist "humic soil" was kept in an atmosphere of nitrogen gas. Warington (*25*) in experiments at Rothamsted in 1880 found that saturating ordinary soil with water caused it to rapidly lose the nitrates it contained. Kellner (*14*) in 1891 and Kelley (*13*) in 1914 found that flooded rice fields contained little or no nitrates.

BACTERIAL NUMBERS

The conditions under which studies of the number of bacteria present in soils have been made have varied to such an extent that generalizations rather than specific correlations have resulted. Chester (*6*) was the first to note that applications of lime increased the bacterial content of soils. He concluded that the favorable action was not

> due to any direct action of the lime, but due to the more favorable reaction which the lime gave the soil.

Later Fabricius and Feilitzen (*10*), Engberding (*9*), and Brown (*5*) reported increased bacterial numbers as the result of liming. Engberding showed that in most cases a lack of lime accounted for low bacterial counts.

Kossowicz (*16*) summarizes the results of investigations by Houston, Th. Remy, Fabricius, and Feilitzen and C. Hoffman, as follows:

> Manuring brings about an increased bacterial content and betters the conditions for the development of those organisms already present in the soil. The time of the year and weather conditions influence the bacterial content of the soil. TRANSLATION.

Koch (*15*), Adametz (*18*) and others have shown that the majority of soil microflora consist principally of rod-shaped organisms. That anaerobic bacteria are present in great numbers has been shown by Ueke (*24*), who found over 13,000,000 anaerobes present in a garden soil.

Löhnis (*18*) states that the multiplication of soil organisms varies with different soil layers, and the number of bacteria present decreases with the depth, air and food being the first considerations.

PRESENT INVESTIGATIONS

Many uncontrolled conditions, such as variations in temperature, moisture, and aeration, are constantly occurring in field practice. The data reported in this paper were obtained in order to ascertain the differences in bacterial numbers, nitrates, and nitrification of five variously treated typical acid soils, after these soils had been kept for 10 months under the same temperatures and controlled moisture conditions in pots where nitrates could not be lost by leaching. The soils used were all very acid and varied widely in organic matter. They were: (1) A yellow silty clay containing 0.7 per cent of humus, 0.07 per cent of nitrogen; (2) a whitish silt loam containing 1.3 per cent of humus, 0.12 per cent of nitrogen; (3) a brown silt loam containing 3.1 per cent of humus, 0.22 per cent of nitrogen; (4) a black peaty sand containing 5 per cent of humus, 0.4 per cent of nitrogen; and (5) a dark-brown peat containing 52 per cent of humus, 2.04 per cent of nitrogen. More complete analyses of these soils and the changes in their acidities due to moisture changes are given by one of us in another paper (*7*).

PREPARATION OF SOILS

To obtain soils for the pot tests, quantities of field soil were taken from the surface 6 inches, sacked, transferred to the station where each soil was mixed over and over without drying, sieved, and potted. Equal weights of a soil were put in galvanized-iron paraffined culture pots 9.25 inches in diameter and 11 inches high. The soil was compacted to that of a good seed bed by dropping the pots a prescribed number of times onto the floor from a height of about 3 inches. The pots were kept in the greenhouse and maintained at the desired moisture contents by weighing two to three times a week and replenishing the evaporated moisture with pure distilled water through an open tube extending from above the surface of the soil to an arch at the bottom of the pot. The surface of the soils of all pots except those kept fully saturated with water was cultivated from time to time to give a very thin dust mulch. The wheat stubble and growing clover were in the pots when sampled. The samples were taken to represent the entire depth of soil in the pot by the use of Noyes' bacteriologists' soil samplers (*19*), and all determinations were made from these samples.[1]

NITRATES AND NITRIFICATION WITH LIME AND FERTILIZER TREATMENTS

The nitrates were determined by the phenoldisulphonic-acid method modified for the accurate determination of soil nitrates.[2] The results are held to be equally accurate for all the soils, since the modified method takes into consideration the obtaining of a clear solution, the presence of soluble salts and interfering organic matter. The nitrification tests were made by the beaker method. One hundred gm. of each soil except the peat, of which 50 gm. were used, were placed in half-pint jelly glasses. Five cc. of a 2 per cent ammonium-sulphate solution were added and the soil was incubated for six weeks at 20° to 21° C. The moisture content at the end of the period of incubation was in every case within 1 per cent of what it was when the soils were sampled. Table I gives the acidity, crop yields, and nitrate data for each soil with the different lime and fertilizer treatments.

The quantities of nitrates found in the untreated soils before incubation showed that nitrification had taken place in every one of the acid soils. The amounts of nitrate present in the untreated soils when sampled were in proportion to their total nitrogen contents rather than in any relation to their acidities. The presence of growing clover in some of the pots lowered the ratio of the nitrates before incubation to those after incubation. Those pots which contained large growths of clover when sampled and which had received applications of lime alone contained less nitrates than the unlimed pots, which contained little or no

[1] The pots used in this investigation were chosen from a series of different investigations on soil-acidity problems, and hence the lime and fertilizer treatments for each soil were not the same.

[2] NOYES, H. A. THE ACCURATE DETERMINATION OF SOIL NITRATES BY THE PHENOL DISULPHONIC-ACID METHOD. To be published in Jour. Indus. and Engin. Chem.

clover. This shows that the nitrates present in the soils were greatly influenced by the growing crop. The limed pot in the brown silt-loam series was no exception to this, as the untreated soil on this series grew good clover. With each soil the amounts of nitrates found after incubation were very much greater with lime than without lime, proving that calcium carbonate promotes nitrification in acid soils. As a rule, the less clover there was per pot the greater the ratio of nitrates before incubation to nitrates after incubation.

TABLE I.—*Effects of lime and fertilization on nitrates and nitrification of five typical acid soils*

Kind of soil and treatment per million pounds of soil.	Acidity.[a]		Crop yields.[b]		Nitrates.			
	Potassium nitrate.	Calcium acetate.	Wheat.	Clover.	Before incubation.	After incubation.	Increase on incubation	Ratio before and after incubation.
YELLOW SILTY CLAY.			*Gm.*	*Gm.*	*P. p. m.*	*P. p. m.*	*P. p. m.*	
No treatment	2,460	4,000	7	0	10	24	14	42
2 tons of calcium carbonate	20	750	10	14	Tr.	32	32	0
Nitrogen, phosphorus, potassium[c]	2,800	4,125	43	2	Tr.	Tr.	0	100
Nitrogen, phosphorus, potassium,[c] 2 tons of calcium carbonate	20	750	68	17	0	184	184	0
Nitrogen, phosphorus, potassium,[c] 6 tons of calcium carbonate	0	500	76	15	0	873	873	0
WHITISH SILT LOAM.								
No treatment	1,360	3,000	23	13	19	38	19	50
3 tons of calcium carbonate	20	500	40	37	17	879	862	2
500 pounds of acid phosphate	1,380	3,000	23	6	29	48	19	60
BROWN SILT LOAM.								
No treatment	460	3,750	19	20	23	92	69	25
3 tons of calcium carbonate	20	750	29	30	52	852	800	6
500 pounds of acid phosphate	460	3,750	20	31	19	119	100	16
BLACK PEATY SAND.								
No treatment	1,800	6,750	0.5	0	350	340	−10	103
2 tons of calcium carbonate	80	3,500	17	11	77	585	508	13
Nitrogen, phosphorus potassium[c]	1,760	6,750	2	1	305	473	168	64
Nitrogen, phosphorus, potassium,[c] 2 tons of calcium carbonate	40	3,000	35	13	52	913	861	6
Nitrogen, phosphorus, potassium,[c] 6 tons of calcium carbonate	10	750	52	14	233	1,280	1,047	18
DARK-BROWN PEAT.								
No treatment	2,040	35,000	0	0	710	710	0	100
2 tons of calcium carbonate	1,260	27,500	0.5	0	1,216	1,280	64	95
20 tons of calcium carbonate	100	9,250	48	14	154	4,736	4,582	3

[a] Acidity determinations were made by Hopkins potassium-nitrate method and C. H. Jones calcium-acetate methods, and expressed in calcium-carbonate requirement per million.

[b] Crop yields are given in grams per pot; average of two pots.

[c] Chemically pure salts: 91 pounds of ammonium nitrate, 72 pounds of ammonium phosphate, and 100 pounds of potassium phosphate on yellow silty clay. No ammonium nitrate was used on black peaty sand.

The yellow silty clay containing 0.07 per cent of nitrogen and the black peaty sand containing 0.40 per cent of nitrogen received the same lime and fertilizer treatments, but gave quite different crop yields, nitrates, and nitrification. These variations can not be entirely correlated with changes in soil acidity. On the yellow silty clay it took both lime and fertilizer to give a markedly increased nitrifying power, while on the black peaty sand, of higher initial nitrifying power, lime gave the large, increased nitrifying power.

The whitish silt loam containing 0.12 per cent of nitrogen received the same lime and acid-phosphate treatment as the brown silt loam containing 0.22 per cent of nitrogen. Lime increased nitrification on both these soils more than acid phosphate did.

SOIL MOISTURE IN RELATION TO NITRATES AND NITRIFICATION

In order to ascertain what effect keeping soils at different moisture contents without crop would have on the nitrates present in the soil and nitrification tests, samples were drawn from a series of pots where each of the five acid soils had been kept at different moisture contents. The nitrates present in the soils after standing 10 months with specified moisture contents are given in Table II.

TABLE II.—*Effects of variable moisture on nitrates and nitrification of five typical acid soils*

Kind of soil and moisture treatments	Acidity[a]		Nitrates.			
	Potassium nitrate	Calcium acetate	Before incubation.	After incubation.	Increase on incubation.	Ratio before and after incubation.
YELLOW SILTY CLAY.			*P. p. m.*	*P. p. m.*	*P. p. m.*	
One-half	3,075	4,500	24	19	−5	126
Full	1,740	3,125	0	0	0	.....
WHITISH SILT LOAM.						
One-fourth	1,550	3,125	136	82	−54	166
One-half	1,860	4,500	74	128	54	58
Full	888	2,750	0	0	0	.....
BROWN SILT LOAM.						
One-fourth	325	4,500	265	100	−165	265
One-half	487	5,000	319	122	−197	261
Full	225	2,500	0	0	0	.....
BLACK PEATY SAND.						
One-fourth	1,560	6,500	140	328	188	43
One-half	1,810	6,250	315	190	−125	166
Full	925	4,750	0	0	0	.....
DARK-BROWN PEAT.						
One-fourth	2,000	31,750	178	214	36	83
One-half	2,700	32,500	618	766	148	81
Full	3,360	34,750	0	0	0	.....

[a] Both methods are expressed in calcium-carbonate requirement per million.

The results given in Table II show that the amount of water present in a soil is concerned with its nitrification, and further, that soils fully saturated with moisture do not contain nitrates either before or after incubation with ammonium sulphate. This table shows even more strongly than Table I that nitrification takes place in an acid soil, for the nitrates contained in the soils when sampled varied directly with the organic matter content of the different soils, but did not increase with lower soil acidities. The many instances where the nitrates in the soil when sampled were greater than those after incubation show that the nitrates present in these uncropped soils were near the maximum that could be present under the conditions of the experiment.

METHOD OF OBTAINING COUNTS

Field conditions are variable, and the results of these variations are apparent in the soil processes, due to bacterial agencies. It was believed that bacterial counts properly made would show some correlations among these different acid soils, the lime and fertilizer treatments, and the variable moisture contents they were kept under. Not only the nitrifying organisms but all classes of organisms had been given 10 months to respond to the different treatments, and an enumeration of both aerobes and anaerobes should show the types of bacteria predominating under the different treatments.

Plate counts were made from plates of high bacterial dilutions of each treatment according to the technic of Noyes and Voigt(*20*). Unpublished work by one of us on aerobic and anaerobic soil bacteria has shown that the average of five plates of a bacterial dilution high enough so that all bacteria from 1 cc. of the dilution will have a chance to develop into colonies in 10 days, gives accordant results. The media used was Lipman and Brown(*17*) modified synthetic agar, which extensive tests have proved to be satisfactory for the development of soil microorganisms. The carbon dioxid and hydrogen incubations were carried out in an atmosphere of flowing hydrogen or carbon-dioxid gas.

AEROBIC AND ANAEROBIC COUNTS ON CROPPED, LIMED, AND FERTILIZED SOILS

The number of bacteria present under the different lime and fertilizer treatments are given in Table III.

Table III shows that large increases in bacterial numbers result from the use of lime. These increases are largely in the aerobic organisms, although with the soils that contain considerable partially decomposed organic matter the anaerobic count is also increased.

Representative aerobic plates obtained from the yellow silty clay are shown in Plate 1. The numbers of colonies per plate are small, allowing for maximum development; yet no striking differences in kinds of microorganisms are apparent under the different treatments. Neither

lime nor complete fertilizer alone had any great influence on bacterial numbers, while complete fertilizer with 2 tons of lime more than doubled the bacterial index (sum of aerobes and anaerobes) of the soil. Six tons of lime with fertilizer did not increase the bacterial index as much as the 2 tons with fertilizer.

TABLE III.—*Effects of lime and fertilization on bacterial content of five typical acid soils*

Kind of soil and treatment per million pounds of soil.	Millions of bacteria per gram of dry soil.		Bacterial index.[a]	Increase of bacterial index due to—	
	Air incubation.	Hydrogen incubation.		Calcium carbonate.	Fertilizer.
YELLOW SILTY CLAY.					
No treatment	[b] 3. 010	0. 100	3. 110	.....	.....
2 tons of calcium carbonate	3. 046	. 381	3. 427	0. 317	.....
Nitrogen, phosphorus, potassium [c]	3. 027	. 000	3. 027	.....	—0. 083
Nitrogen, phosphorus, potassium,[c] 2 tons of calcium carbonate	7. 605	. 000	7. 605	4. 578	4. 178
Nitrogen, phosphorus, potassium,[c] 6 tons of calcium carbonate	5. 244	. 000	5. 244	2. 217	.....
WHITISH SILT LOAM.					
No treatment	5. 021	1. 545	6. 566	.....	.....
3 tons of calcium carbonate	14. 810	. 898	15. 708	9. 142	.....
500 pounds of acid phosphate	5. 531	. 000	5. 531	.....	—1. 035
BROWN SILT LOAM.					
No treatment	9. 904	. 189	10. 093	.....	.....
3 tons of calcium carbonate	23. 921	2. 556	26. 477	16. 384	.....
500 pounds of acid phosphate	11. 161	2. 714	13. 878	.....	3. 785
BLACK PEATY SAND.					
No treatment	3. 146	1. 154	4. 300	.....	.....
2 tons of calcium carbonate	8. 386	1. 617	10. 003	5. 703	.....
Nitrogen, phosphorus, potassium [b]	2. 813	2. 907	5. 720	.....	1. 420
Nitrogen, phosphorus, potassium, 2 tons of calcium carbonate	10. 583	. 099	10. 682	4. 962	. 679
Nitrogen, phosphorus, potassium, 6 tons of calcium carbonate	16. 037	1. 760	17. 797	12. 077	.....
DARK-BROWN PEAT.					
No treatment	1. 554	. 997	2. 551	.....	.....
2 tons of calcium carbonate	3. 420	1. 440	4. 860	2. 309	.....
20 tons of calcium carbonate	91. 846	11. 752	103. 598	101. 047	.....
Average	12. 109	1. 585	13. 694	15. 874	.....

[a] Sum of air and hydrogen counts.
[b] Average of five plates. No count indicates no colonies on plates from 1:400,000 bacterial dilutions.
[c] Chemically pure salts: 91 pounds of ammonium nitrate, 72 pounds of ammonium phosphate, and 100 pounds of potassium phosphate on yellow silty clay. No ammonium nitrate was used on black peaty sand.

Lime more than doubled the bacterial indexes of the whitish silt and brown silt loams. Acid phosphate decreased the anaerobic counts of the whitish silt loam enough more than it increased the aerobic con-

tents so that the bacterial index was decreased. With the brown silt loam containing considerable undecayed organic matter the acid phosphate increased both the aerobic and anaerobic counts somewhat. Plate 2 shows representative petri plates from each treatment for the two soils. This plate shows a marked similarity between the colonies on the aerobic plates from the limed pots of both soils. The similarity of the appearances of the plates from the limed and the phosphated pots of the whitish silt loam, the similarity of all aerobic plates from the brown silt loam and the uniformity of colonies developing from the brown silt loam under anaerobic conditions are to be noted.

The black peaty sand containing six times as much nitrogen as the yellow silty clay, received the same lime and fertilizer treatments as the yellow clay, but gives entirely different results. Lime and fertilizer both alone and in combination give increased bacterial indexes. While the aerobic organisms are increased by lime, the organic matter of the black peaty sand must be in an advanced stage of decay since the counts are lower than they should be if the organic matter was good food for bacteria. Plate 3 shows representative culture plates from this soil. These illustrations emphasize the effect of lime on bacterial numbers and the small proportion of the bacteria which are anaerobic.

The dark-brown peat shows an increase of over 100,000,000 in bacterial index as the result of liming. Peats *in situ* are generally low in bacterial content. Working them over after drainage generally causes enormous increases in their bacterial content. This peat, even when aerated, had only 1½ times as many aerobic as anaerobic bacteria, but adequate liming increased the aerobes more than 60 times and the anaerobes over 11 times. The increase in anaerobes is believed to be associated with the large amount of organic matter present in the soil. Plate 4 shows representative petri plates of the colonies developing in air and hydrogen. Attention is called to the small variation in colony types on the anaerobic plates as compared to the aerobic. The aerobic culture plates from the heavily limed soil showed many chromogenic differences between colonies not observable in the photographs.

SOIL MOISTURE IN RELATION TO BACTERIAL COUNTS

In addition to the incubations in air and hydrogen another set of plates was incubated in an atmosphere of flowing carbon-dioxid gas for 10 days. No colonies developed on this set of plates while they were in carbon-dioxid gas. The counts given were computed from colonies developing in 10 days in air after the plates had been removed from the carbon dioxid.[1] Table IV gives the counts under the different conditions of incubation and the various soil-moisture contents.

[1] These organisms, as far as tested, have been found to be spore formers.

TABLE IV.—*Effects of variable moisture on bacterial content of five typical acid soils*

Kind of soil and degree of moisture saturation.	Millions of bacteria per gram of dry soil.			Bacterial index.[b]	Ratios to bacterial index as 100.		
	Air.	Hydrogen.	Carbon-dioxid-surviving.[a]		Air.	Hydrogen.	Carbon-dioxid-surviving.
YELLOW SILTY CLAY.							
One-half	[c] 1.556	0.101	0.131	1.657	.94	6	8
Full	.184	.032	.075	.216	85	15	35
WHITISH SILT LOAM.							
One-fourth	2.792	.367	.282	3.159	88	12	9
One-half	3.688	1.171	.216	4.859	76	24	4
Full	3.179	.353	.246	3.532	90	10	7
BROWN SILT LOAM.							
One-fourth	4.920	1.879	.533	6.799	72	28	8
One-half	4.513	1.640	.847	6.153	73	27	14
Full	7.854	2.864	.575	10.718	73	27	5
BLACK PEATY SAND.							
One-fourth	1.641	.453	.286	2.094	78	22	14
One-half	2.363	1.270	.463	3.633	65	35	13
Full	3.316	.129	1.071	3.445	96	4	31
DARK-BROWN PEAT.							
One-fourth	2.425	1.988	1.101	4.413	55	45	25
One-half	1.796	1.914	1.204	3.710	48	52	33
Full	2.257	.735	.499	2.992	75	25	17
Averages	3.034	1.064	.538	4.098	74	26	13

[a] Incubated 10 days in carbon dioxid; then 10 days in air.
[b] Sum of air and hydrogen incubation.
[c] All figures were computed from 5 plates.

The bacterial content, as well as the proportions of aerobes to anaerobes, was changed by the degree of saturation of the soil, but the nature of the soil had a greater effect than the moisture content on bacterial numbers. The proportions of anaerobes to the aerobes which survived carbon-dioxid incubation increased with soil organic matter when the soils were held under optimum moisture conditions.

Plates 5 to 9 show representative petri plates from the 1 to 40,000 bacterial dilution of these soils. Figures A_1, H_1, and C_1 in each plate show representative petri plates after air (A), hydrogen (H), and carbon-dioxid, then air (C) incubations of bacterial dilutions of samples from pots of soils kept one-fourth saturated with water. Figures A_2, H_2, and C_2 are from samples from pots of soils kept half saturated, while A_3, H_3, and C_3 are from pots of soils kept fully saturated with water.

Plates 5 to 9 show that the bacterial flora of each soil is different from that of every other soil. The soils kept one-fourth saturated with water

contained the largest numbers of microorganisms developing moldlike colonies, and the fully saturated soils gave culture plates containing the smallest numbers of spreading moldlike colonies.

NITRATES AND NITRIFICATION IN RELATION TO BACTERIAL COUNTS

Lime increased both nitrification and bacterial counts. A study of Plates 1 to 4 shows that the increases in bacterial numbers can be asso-

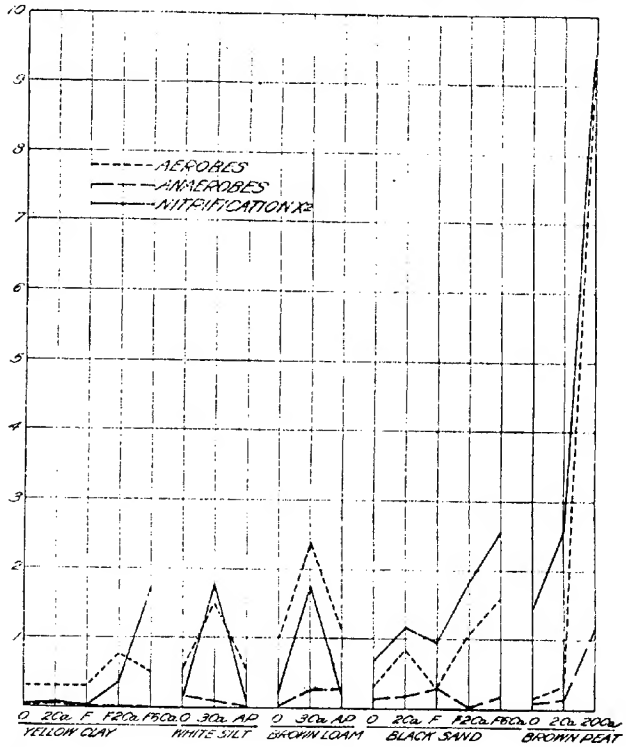

FIG. 1.—Graphs showing the relation of aerobes and anaerobes to nitrification of five acid soils with and without lime and fertilizer treatments.

ciated principally with the aerobic small, round, entire colonies on the petri plates. Figure 1 shows the relations between aerobic and anaerobic bacteria and the nitrates after incubation for the cropped, limed, and fertilized soils kept at optimum moisture content. The nitrates after incubation varied directly with the aerobic bacteria. The aerobic count and nitrates after incubation show that it is the increased number of aerobic organisms that are to be associated with increased nitrification.

Figure 2 gives soil nitrates, aerobic, and anaerobic bacterial numbers for the series of soils where moisture was the variable. These graphs shows that lack of aeration which changed the proportions of aerobes to anaerobes prevented a correlation between nitrates and aerobic counts.

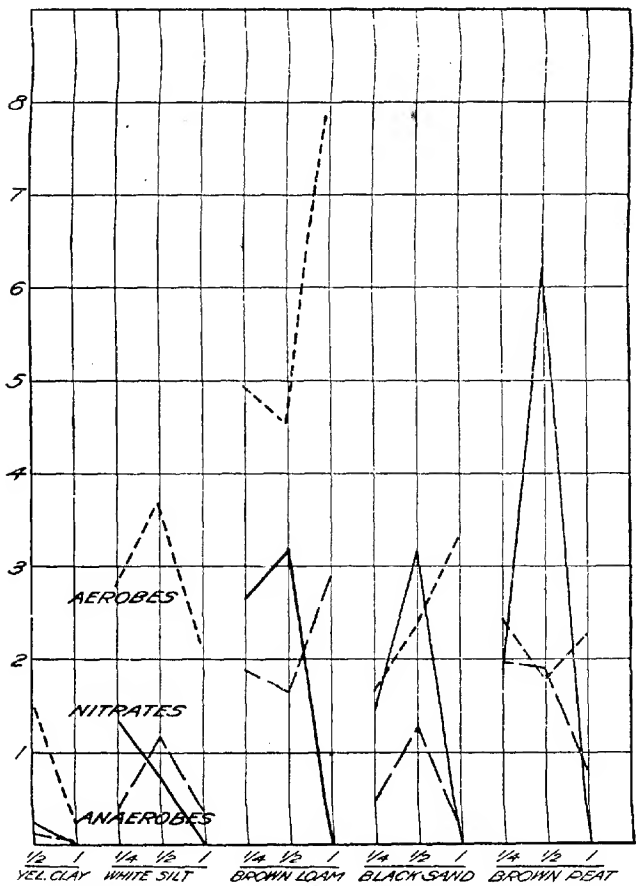

FIG. 2.—Graphs showing the relation of aerobes and anaerobes to nitrates in five acid soils kept at different moisture contents.

GENERAL DISCUSSION

After conducting bacteriological investigations on acid soils to ascertain "if it might be desirable to consider more carefully the possibilities of a system of acid agriculture," Bear (3) concluded that—

> the supply of nitrogen in acid soils may be maintained by growing acid-resistant legumes, of which the soy bean is one. Undoubtedly the use of acid phosphate aids

materially in the nitrogen-fixation processes of acid soils. Small applications of calcium carbonate are, as a rule, relatively more effective than large applications as a means of increasing the bacterial activities in acid soils.

The problem of maintaining soil fertility resolves itself into maintaining and increasing the available supply of organic matter and nitrogen in the soil and the replenishing of the mineral elements. One system now generally recommended and used is to apply lime and phosphates, then to grow legumes, and to plow them under. This system of soil maintenance and improvement is in accordance with the important rôle of soil bacteria in plant nutrition, and the results obtained in the controlled investigations reported here illustrate some good reasons for such a method of soil management.

When the soil was limed, the aerobic bacteria concerned with oxidation reactions increased in numbers. This is illustrated by the increased bacterial numbers and nitrification wherever the soils were limed.

Plenty of organic matter is necessary for high bacterial numbers, a condition which is well illustrated by the low bacterial content and nitrate results with the limed yellow silty clay (low in organic matter) compared with the high bacterial contents and nitrates on the limed brown silt loam and dark-brown peat (high in organic matter).

Mineral fertilizers serve as food for larger crops and larger crops in turn leave more residues in roots and stubble for bacterial food.

The number of bacteria in an arable soil can be correlated with crop yield to about the same degree that soil moisture can be. Soil moisture is conceded to be the most vital single factor influencing crop yields; yet because of so many other variable conditions it is not always possible to correlate soil moisture and crops any more than it is possible to always correlate bacterial numbers and crops. Below a certain minimum in moisture or bacterial numbers field soils will not produce crops; above that minimum, everything else being equal, crops may be in general correlated with bacterial numbers as well as with moisture.

Changes in bacterial numbers, especially differences in the proportions of aerobes to anaerobes, are of prime importance in soil-biology studies. The results here reported under controlled conditions make it evident that soil-fertility investigations should include both chemical and biological examinations of the soil.

SUMMARY

(1) Controlled greenhouse investigations were conducted on five typical acid soils. In part of the experiments the soils were fertilized with calcium carbonate, acid phosphate, and complete fertilizer, cropped to wheat and clover, and kept at optimum moisture content, while in another series the soils were unfertilized, uncropped, and kept one-fourth, one-half, and fully saturated with water.

(2) The results reported include crop yields, soil-acidity determinations, nitrates in the soil when sampled and after incubation with ammo-

nium sulphate, and also the numbers of aerobic, anaerobic, and carbon-dioxid surviving microorganisms present in the soils.

(3) All the untreated soils were quite acid and contained nitrates when sampled, showing that nitrification takes place in acid soils.

(4) The amounts of nitrates present and the nitrifying power of the untreated acid soils varied with the organic matter and total nitrogen rather than with the soil acidity.

(5) Calcium-carbonate additions markedly increased the nitrification of all five soils.

(6) Fertilization tended to increase nitrification, but not so much as calcium carbonate did.

(7) Regardless of treatments the presence of growing clover kept down nitrate contents of the soils.

(8) The degree of saturation of the soils affected the nitrates present. As a rule, more nitrate were found in soil kept one-half saturated with water than in soil kept one-fourth saturated.

(9) The soils that had been kept fully saturated with water for the 10 months contained no nitrates and formed no nitrates when incubated with ammonium sulphate.

(10) The relation of nitrates present in the uncropped soils before incubation to the nitrates present after incubation shows that the nitrate contents of these acid soils tend to reach an equilibrium, above which no increase is obtained without additional treatment.

(11) The bacterial flora of each soil was different from that of every other soil.

(12) No bacteria developed into colonies visible to the eye as long as plates were incubated in an atmosphere of flowing carbon-dioxid gas.

(13) Calcium-carbonate additions increased the bacterial contents of the soils. This increase was largely in the aerobic organisms.

(14) Small increases in bacterial content resulted from the use of fertilizer.

(15) The degree of saturation at which the soil was kept changed the proportions between the aerobic, anaerobic, and carbon-dioxid-surviving bacteria.

(16) Cultures from samples that had been kept one-fourth saturated with water contained the largest proportions of organisms forming moldlike colonies.

(17) Under optimum moisture conditions both without and with lime and fertilizer treatments the nitrates after incubation varied directly with the aerobic counts.

(18) In general, the greater the aerobic bacterial content and the nitrifying power of the soil the larger the crop yields.

(19) These investigations show many reasons why a system of soil improvement which includes the addition of lime, phosphate, and organic matter is worth while.

(20) It is evident that soil fertility investigations should include both chemical and biological examinations of the soil.

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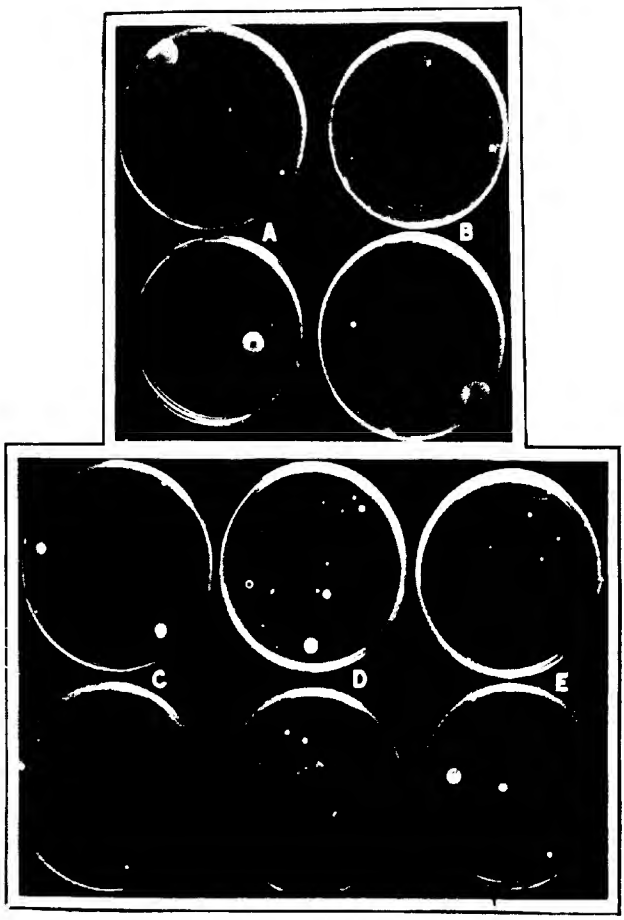
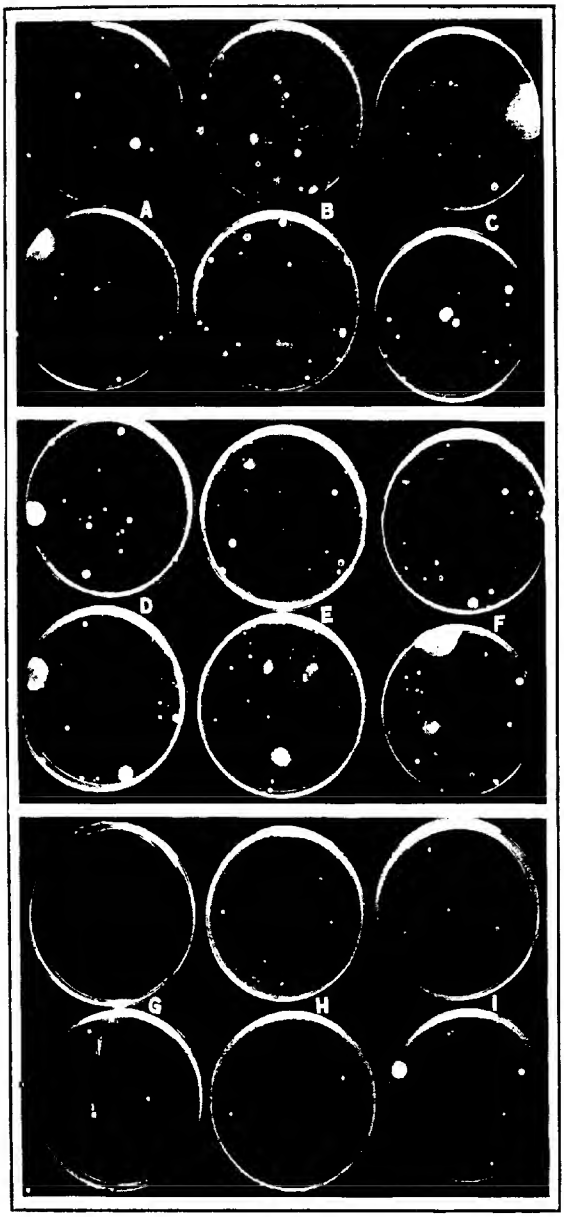
PLATE 1

Representative plates from 1 to 400,000 bacterial dilution of acid yellow silty clay, cropped and held under optimum moisture conditions:

A.—Aerobic plates, untreated.
B.—Aerobic plates, treated with 2 tons of calcium carbonate.
C.—Aerobic plates, treated with complete fertilizer.
D.—Aerobic plates, treated with complete fertilizer and 2 tons of calcium carbonate.
E.—Aerobic plates, treated with complete fertilizer and 6 tons of calcium carbonate.


A
B
C
D
E


A
B
C
D
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F
G
H
I

PLATE 2

Representative plates from 1 to 400,000 bacterial dilution of acid whitish silt loam and acid brown silt loam cropped and held under optimum moisture conditions:

A.—Aerobic plates, acid whitish silt loam, untreated.

B.—Aerobic plates, acid whitish silt loam, treated with 3 tons of calcium carbonate.

C.—Aerobic plates, acid whitish silt loam, treated with 500 pounds of acid phosphate.

D.—Aerobic plates, acid brown silt loam, untreated.

E.—Aerobic plates, acid brown silt loam, treated with 3 tons of calcium carbonate.

F.—Aerobic plates, acid brown silt loam, treated with 500 pounds of acid phosphate.

G.—Anaerobic plates, acid brown silt loam, untreated.

H.—Anaerobic plates, acid brown silt loam, treated with 3 tons of calcium carbonate.

I.—Anaerobic plates, acid brown silt loam, treated with 500 pounds of acid phosphate.

PLATE 3

Representative plates from 1 to 400,000 bacterial dilution of acid black peaty sand, cropped and held under optimum moisture conditions:

A.—Aerobic plates, untreated.

B.—Aerobic plates, treated with 2 tons of calcium carbonate.

C.—Aerobic plates, treated with complete fertilizer.

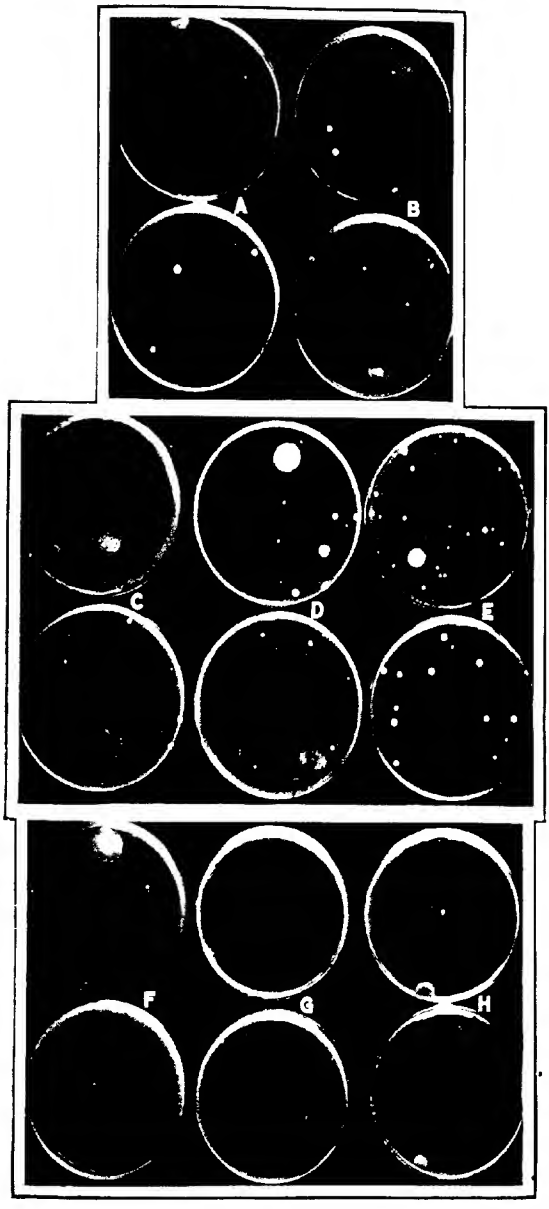
D.—Aerobic plates, treated with complete fertilizer and 2 tons of calcium carbonate.

E.—Aerobic plates, treated with complete fertilizer and 6 tons of calcium carbonate.

F.—Anaerobic plates, treated with complete fertilizer.

G.—Anaerobic plates, treated with complete fertilizer and 2 tons of calcium carbonate.

H.—Anaerobic plates, treated with complete fertilizer and 6 tons of calcium carbonate.


A
B
C
D
E
F
G
H


A
B
C
D
E
F

PLATE 4

Representative plates from 1 to 400,000 bacterial dilution of acid dark-brown peat, cropped and held under optimum moisture conditions:

A.—Aerobic plates, untreated.
B.—Aerobic plates, treated with 2 tons of calcium carbonate.
C.—Aerobic plates, treated with 20 tons of calcium carbonate.
D.—Anaerobic plates, untreated.
E.—Anaerobic plates, treated with 2 tons of calcium carbonate.
F.—Anaerobic plates, treated with 20 tons of calcium carbonate.

PLATE 5

Representative plates from 1 to 40,000 bacterial dilution of acid yellow silty clay kept at different moisture contents:

A_2.—Aerobic plates, from soil kept one-half saturated.

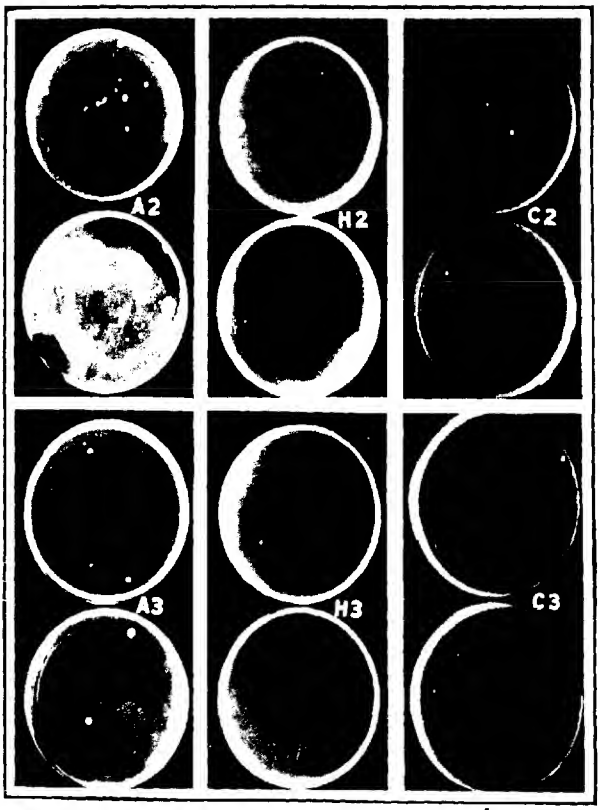
H_2.—Anaerobic plates, from soil kept one-half saturated.

C_2.—Aerobic plates of carbon-dioxid-surviving organisms from soil kept one-half saturated.

A_3.—Aerobic plates from soil kept fully saturated.

H_3.—Anaerobic plates from soil kept fully saturated.

C_3.—Aerobic plates of carbon-dioxid-surviving organisms from soil kept fully saturated.


A2
H2
C2
A3
H3
C3

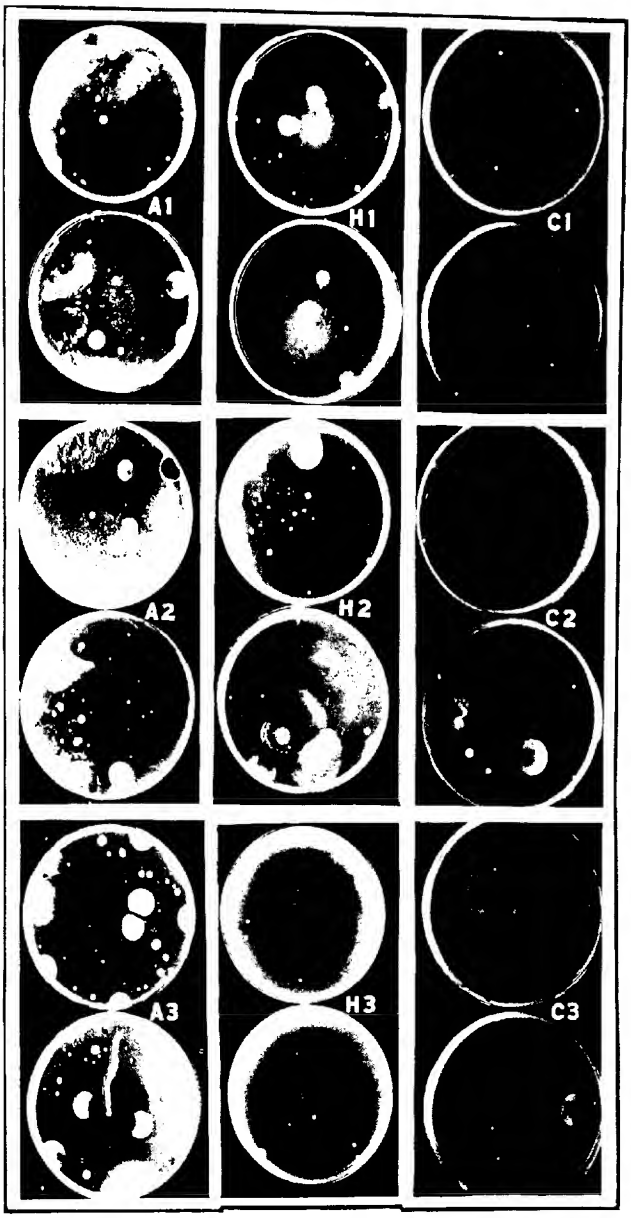

A1
H1
C1
A2
H2
C2
A3
H3
C3

PLATE 6

Representative plates from 1 to 40,000 bacterial dilution of acid whitish silt loam kept at different moisture contents:

A_1.—Aerobic plates from soil kept one-fourth saturated.

H_1.—Anaerobic plates from soil kept one-fourth saturated.

C_1.—Aerobic plates of carbon-dioxid-surviving organisms from soil kept one-fourth saturated.

A_2.—Aerobic plates from soil kept one-half saturated.

H_2.—Anaerobic plates from soil kept one-half saturated.

C_2.—Aerobic plates of carbon-dioxid-surviving organisms from soil kept one-half saturated.

A_3.—Aerobic plates from soil kept fully saturated.

H_3.—Anaerobic plates from soil kept fully saturated.

C_3.—Aerobic plates of carbon-dioxid-surviving organisms from soil kept fully saturated.

PLATE 7

Representative plates from 1 to 40,000 bacterial dilution of acid brown silt loam kept at different moisture contents:

A_1.—Aerobic plates from soil kept one-fourth saturated.

H_1.—Anaerobic plates from soil kept one-fourth saturated.

C_1.—Aerobic plates of carbon-dioxid-surviving organisms from soil kept one-fourth saturated.

A_2.—Aerobic plates from soil kept one-half saturated.

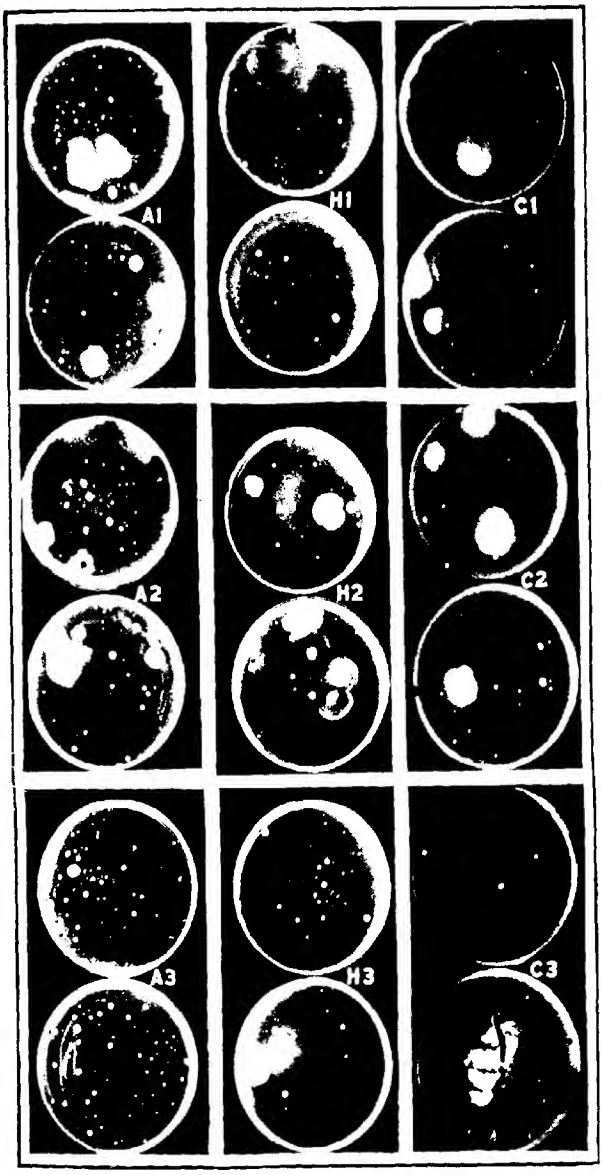
H_2.—Anaerobic plates from soil kept one-half saturated.

C_2.—Aerobic plates of carbon-dioxid-surviving organisms from soil kept half saturated.

A_3.—Aerobic plates from soil kept fully saturated.

H_3.—Anaerobic plates from soil kept fully saturated.

C_3.—Aerobic plates of carbon-dioxid-surviving organisms from soil kept fully saturated.


A1
H1
C1
A2
H2
C2
A3
H3
C3

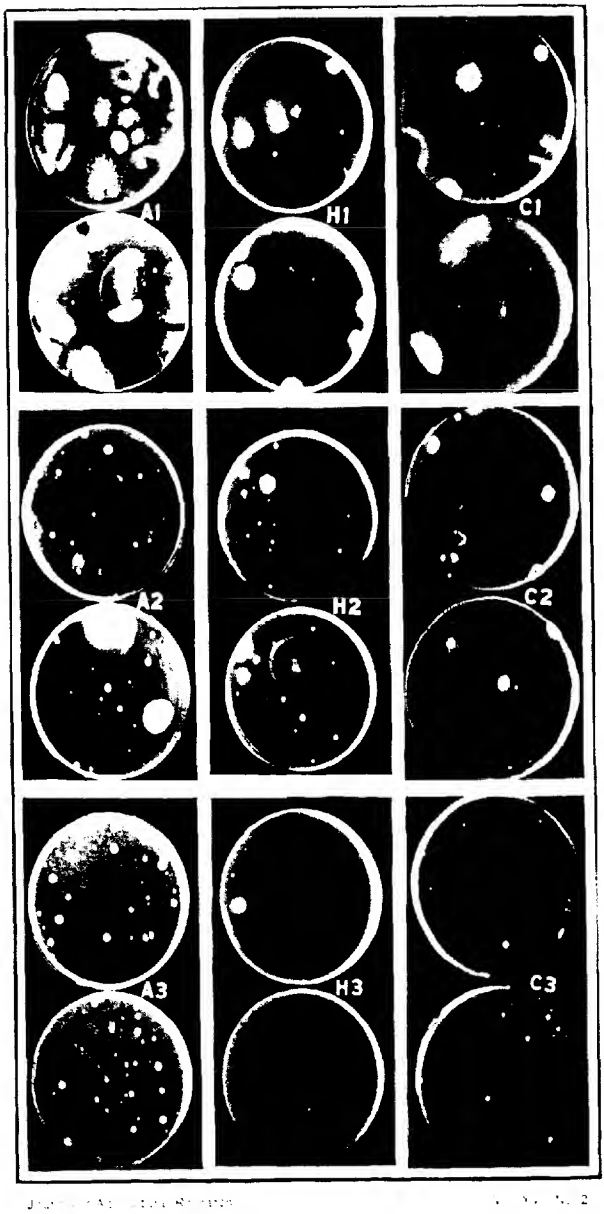

A1
H1
C1
A2
H2
C2
A3
H3
C3

PLATE 8

Representative plates from 1 to 40,000 bacterial dilution of acid black peaty sand kept at different moisture contents:

A_1.—Aerobic plates from soil kept one-fourth saturated.

H_1.—Anaerobic plates from soil kept one-fourth saturated.

C_1.—Aerobic plates of carbon-dioxid-surviving organisms from soil kept one-fourth saturated.

A_2.—Aerobic plates from soil kept one-half saturated.

H_2.—Anaerobic plates from soil kept one-half saturated.

C_2.—Aerobic plates of carbon-dioxid-surviving organisms from soil kept one-half saturated.

A_3.—Aerobic plates from soil kept fully saturated.

H_3.—Anaerobic plates from soil kept fully saturated.

C_3.—Aerobic plates of carbon-dioxid-surviving organisms from soil kept fully saturated.

PLATE 9

Representative plates from 1 to 40,000 bacterial dilution of acid dark-brown peat kept at different moisture contents:

A_1.—Aerobic plates from soil kept one-fourth saturated.

H_1.—Anaerobic plates from soil kept one-fourth saturated.

C_1.—Aerobic plates of carbon-dioxid-surviving organisms from soil kept one-fourth saturated.

A_2.—Aerobic plates from soil kept one-half saturated.

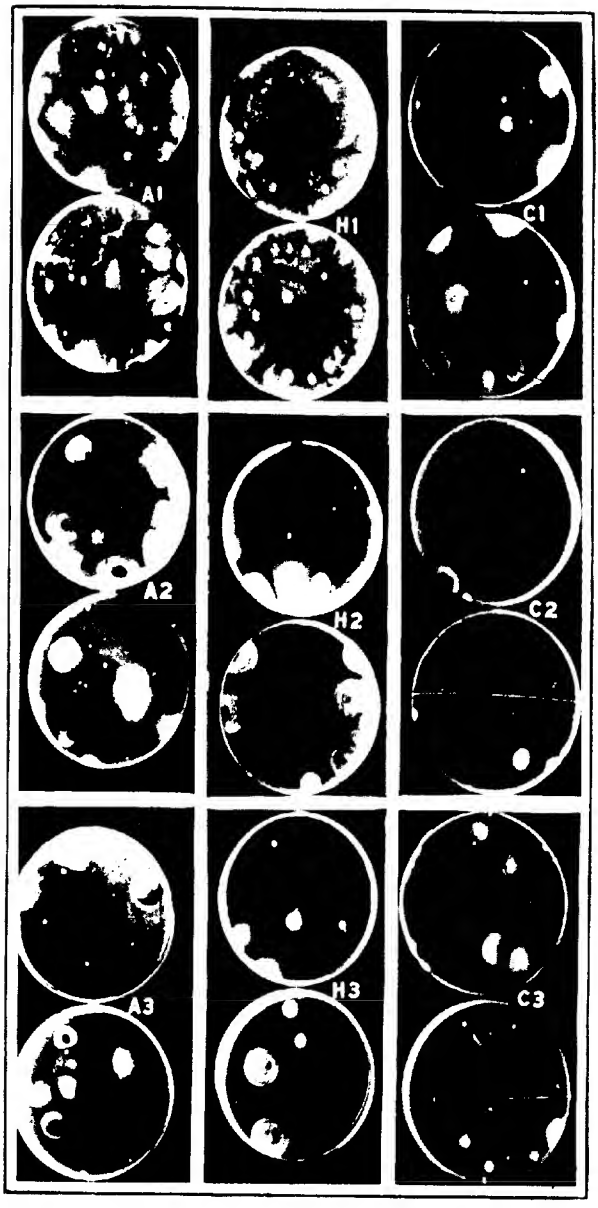
H_2.—Anaerobic plates from soil kept one-half saturated.

C_2.—Aerobic plates of carbon-dioxid-surviving organisms from soil kept one-half saturated.

A_3.—Aerobic plates from soil kept fully saturated.

H_3.—Anaerobic plates from soil kept fully saturated.

C_3. Aerobic plates of carbon-dioxid-surviving organisms from soil kept fully saturated.


A1
H1
C1
A2
H2
C2
A3
H3
C3

EFFECT OF CERTAIN ECOLOGICAL FACTORS ON THE MORPHOLOGY OF THE UREDINIOSPORES OF PUCCINIA GRAMINIS [1]

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COOPERATIVE INVESTIGATIONS BETWEEN THE AGRICULTURAL EXPERIMENT STATION OF THE UNIVERSITY OF MINNESOTA AND THE BUREAU OF PLANT INDUSTRY OF THE UNITED STATES DEPARTMENT OF AGRICULTURE

INTRODUCTION

Extensive studies have been made of biologic forms of *Puccinia graminis* Pers., but these studies have been mainly on the physiological rather than on the morphological phase of the problem. The effect of host plants and other factors on the parasitic capabilities of biologic forms has been quite thoroughly investigated. Some work has also been done on the effect of host plants on the morphology of the fungus, but hardly as much as the importance of the problem warrants.

The question whether biologic forms change readily in response to environmental conditions is important practically and scientifically. The measure of plasticity has usually been the parasitic performance of the rust. But if there is a tendency for biologic forms to change rather quickly, it is reasonable to expect that the morphology might change also. The object of this work, therefore, was to determine the effect of hosts and of physical factors such as heat, light, and humidity on the morphology or urediniospores. It would be desirable to include a study of teliospores and æciospores also, but the difficulties are obvious.

Since the effect of physical factors on the morphology of the urediniospores may be indirect—by affecting the vigor of the rust—the virulence of the rust under different conditions was also studied.

Although it has been generally believed that the various biologic forms of *P. graminis* differ only functionally, yet as early as 1902, Ward (*15, p. 236*) [2] suggested that each specialized form—

> is in course of becoming a species and may during the lapse of time actually become a species of Puccinia, which will eventually show morphological differences in addition to the physiological ones it already shows.

Freeman and Johnson (*3, p. 14*) expressed a similar opinion in 1911. Stakman also (*11*) obtained some evidence that long association with

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[2] Reference is made by number (italic) to "Literature cited," p. 77.

a given host might change the urediniospore dimensions of a biologic form.

Recently Long (*8*) working with *Puccinia ellisiana* Thuem. and *P. andropogonis* Schw., whose æcial hosts are certain species of Viola and Pentstemon, respectively, found he could change the morphological characters of the urediniospores of these rusts by reversing their æcial hosts. Thus *P. ellisiana* after passing through *Pentstemon* as the æcial host acquired the morphological characteristics of the urediniospores of *P. andropogonis;* likewise, *P. andropogonis* assumed the characters of *P. ellisiana* after passing through *Viola* sp. as the æcial host.

Attention has been called several times to the fact that when a biologic form of stemrust develops weakly on a partly resistant host the urediniospores usually are appreciably smaller than they normally are. It seems, therefore, that physical factors might also influence the spore size by affecting the vigor of the rust. But, although a great deal of work has been done on the effect of environmental factors on the severity of rust attacks, the possible correlation between the degree of vigor of the rust fungus and the size of the spores has not been investigated thoroughly.

Ward (*15, p. 274*) noted that differences of temperature, illumination, drouth, etc., affecting the transpiration, assimilation, and other processes of the seedlings, also affect the period of germination, incubation and maturation of the rusts. Fromme (*4, p. 507–509*) has tabulated a number of recorded observations of this nature.

Johnson (*7, p. 47*) found the cardinal temperature of *Puccinia graminis* on wheat, barley, and oats to vary from about 35 to 90° F. Butler and Hayman (*1, p. 11*) have not succeeded in producing rust artificially on plants grown in the open in the hot weather, in India, and they doubted—

> whether the spores have power to infect when exposed to temperatures exceeding 100° F.

Christman (*2, p. 106*)—

> found by experiment that [in Wisconsin] in the cooler weather of spring the incubation period following inoculation with uredospores is usually lengthened to between three and four weeks.

Although there was considerable evidence on the effect of these environmental factors on rust, their effect was investigated again, especially for the purpose of getting evidence of the effect on spore morphology.

EXPERIMENTAL METHODS

The methods employed in these experiments were essentially the same as those described by Stakman and Piemeisel (*14*). But it was thought advisable to obtain additional data on the following points:

(1) Quantity of inoculum to be used; (2) optimum length of incubation; (3) condition of urediniospores necessary to insure uniform measurements; (4) number of measurements to be made of a given strain; and (5) method of computation to be employed.

In order to determine the amount of inoculum to be used, eight sets of inoculations were made with very heavy, with moderate, and with exceedingly light applications of inoculum. The very heavily inoculated plants produced 132 successful infections out of 142 inoculations, or 93 per cent; 130 out of 142, or 91 per cent, of the leaves inoculated with a moderate amount became infected; and 104 out of 118, or 88 per cent, of those which had received a small amount of inoculum became infected. Whenever infection resulted, there was no perceptible difference in the size of either the uredinia or the urediniospores, or in the virulence of attack in general.

A liberal amount of inoculum was used whenever possible in all subsequent experiments.

Jaczewski (*5, p. 330*) found that the germination of urediniospores begins two or three hours after placing them in water or on the surface of the plant blades, after which it progresses very rapidly, provided the spores are fresh. Fromme (*4, p. 513*) points out that in order to obtain a successful infection on plants a saturated atmosphere is necessary.

To determine the optimum length of the incubation period, wheat plants in 10 pots, each containing 10 wheat seedlings 6 days old, were inoculated with an equal and liberal amount of viable urediniospore material of *P. graminis tritici* and placed in two pans containing a small amount of water and then placed under glass bell jars. Equal amounts of water were put in both pans, and all other conditions were kept uniform.

At the end of 12 hours two pots were removed from under the bell jars and set out on the bench; after 24 hours the second pair of pots were set out; and the rest were taken out from the pans in pairs every 24 hours following the second pair—that is, 48, 72, and 96 hours after inoculation.

The first observation was made 80 hours after inoculation, and no signs of infection could be detected on the plants incubated for 12, 24, or 48 hours; but 6 plants of those which had been under for 72 hours and 12 of those that were still under the bell jar showed very indistinct, but apparently typical, rust flecks. These were later found not to be infection flecks, but the result of supersensibility, due to the long confinement in the moist chambers. At 128 hours after inoculation clearly defined rust flecks appeared on all plants which had been incubated for 48, 72, and 96 hours. Of the 20 plants that had been under the bell jar for 24 hours, 18 plants were flecked, while only a single fleck showed on one plant of those that had been under only 12 hours.

The first uredinia began to burst through the epidermis 144 hours after inoculation, except on those plants which had been under the bell jar for 12 hours. On these plants the first and only uredinium appeared 10 days after inoculation. At this time the rust was well developed on all the plants that had been under for 48 hours, whereas those that were kept under 24, 72, and 96 hours showed the maximum infection only two days later.

Although all the fully developed uredinia were in every case approximately the same size, color, and shape, the virulence of the attack varied considerably. The plants kept under the bell jar for 48 hours produced the greatest number of uredinia per leaf; those which had been under 24 and 72 hours, respectively, somewhat fewer, and those which had been under 96 hours, still fewer; while only one uredinium appeared on the single infected leaf of the plants kept under for 12 hours. In the present work, therefore, all inoculated plants were kept for 48 hours in the moist chamber, then removed to their respective places on the greenhouse benches.

It was found that the superficial layer of each uredinium contains larger spores, and when this layer is removed, the remaining spores are considerably smaller. But if the uredinium is allowed to produce a new crop of spores, those on the surface again attain the same dimensions as the original ones. For this reason precaution was taken to measure spores from uredinia in the same stage of development.

TABLE I.—*Results of measuring varying numbers of urediniospores of Puccinia graminis tritici and P. graminis avenae*

Experiment No.	Source of urediniospores.	Number of spores measured.	Spore dimensions.		
			Range of length.	Range of width.	Modes.
			μ	μ	μ
1	*Triticum aestivum*	25	26. 88–38. 72	17. 92–22. 08	[a]32. 00×19. 52
2	do	50	26. 88–40. 32	17. 92–23. 04	[b]33. 60×19. 84
3	do	100	26. 56–40. 32	16. 96–23. 36	32. 96×19. 84
4	do	200	25. 60–40. 32	16. 32–23. 36	32. 96×19. 84
5	do	400	23. 68–40. 32	16. 32–23. 36	32. 96×19. 84
6	*Avena sativa*	25	23. 36–35. 20	17. 28–22. 08	[c]
7	do	50	23. 30–35. 20	17. 28–22. 08	[b]27. 52×19. 52
8	do	100	23. 04–35. 20	16. 96–22. 08	29. 12×19. 52
9	do	200	21. 12–36. 48	16. 32–23. 04	29. 12×19. 52

[a] Modes doubtful but showing tendency to form as indicated.
[b] Mode of length doubtful but that of width definitely established.
[c] Modes indeterminate.

As to the number of spores to be measured from a given group, it was found that 100 gave equally as good results as 200 or 400, while when less than 100 were used the results were not always representative or conclusive. Table I gives the results of measuring different numbers of

spores from the same plant taken on the same day from uredinia on the same leaves. Stemrust of both wheat and oats was tried with similar results. As noted from Table I, the modes of a population of 100, 200, or 400 spores are the same, but the limits of variation are less in a population of 100 than in those of 200 or 400. It will also be seen from the table that at least 100 spores should be used. In the present investigation 200 spore measurements were made for each experimental group until January 1, 1916, which constituted about half of all measurements made. Beginning with this date 100 spore measurements for length and 100 for width were made, instead of 200 for each.

As a comparative basis of dimensions in this work, the biometric mode is used in preference to the arithmetic mean. The mode represents the group containing the largest number of individuals of a certain size, thus indicating that this size is the prevailing one in a given spore population. Comparative calculations made show that, as a general rule, arithmetic means usually fluctuate around the biometric modes, as seen from Table II; and consequently there is, on the whole, but little difference between the two bases of recording. It will be seen that in most cases the figures are almost identical; in two cases they are the same; and only in one case is there a difference of 0.24 μ, which may be considered negligible, since two consecutive measurements of the same group of spores may give even greater variation. In this experiment 100 spores were measured in each case.

TABLE II.—*Correlation of biometric modes with arithmetic means of urediniospore dimensions of Puccinia graminis tritici on wheat*

Number of generations rust was confined to wheat.	Biometric modes.	Arithmetic means.
	μ	μ
1	33.28×19.84	33.36×19.69
7	32.00×19.84	31.58×19.84
10	32.64×20.16	32.64×20.02

The apparatus used for meteorological observations is fully described in the discussions of the particular experiments performed. General notes on the behavior of the various cultures were taken at the close of each urediniospore generation before transfers were made to new plants, on the average every two or three weeks. The preliminary spore measurements were made of the original rusts found on the grasses in the field, the subsequent measurements were made on the first following generation and once or twice more during the period the rust was kept in culture. For color determination Ridgway's[1] chromotaxia was used. The Zeiss screw micrometer was used for measuring the urediniospores.

[1] RIDGWAY, Robert. COLOR STANDARDS AND COLOR NOMENCLATURE. 43 p., 53 col. pl. Washington, D. C.

MORPHOLOGY OF BIOLOGIC FORMS STUDIED

The following biologic forms were investigated: *Puccinia graminis tritici* Erikss. and Henn., *P. graminis tritici-compacti* Stak. and Piem., *P. graminis secalis* Erikss. and Henn., *P. graminis avenae* Erikss. and Henn., *P. graminis phleipratensis* (Erikss. and Henn.) Stak. and Piem. and *P. graminis agrostis* Erikss.

It has been stated by Stakman and Piemeisel (*14, p. 484*) that—

> In general, the size and shape of urediniospores of different biologic forms of *Puccinia graminis* are similar. If, however, large numbers of spores are measured and the arithmetical mean or biometrical mode is determined, it becomes quite apparent that there are appreciable and fairly constant differences, provided the spores measured be taken from congenial hosts.

This was substantiated by the writers by many thousands of spore measurements and careful computations. It is necessary, however, to maintain uniform cultural conditions, since the range of variability in size of urediniospores under different conditions is sufficiently great to cause overlapping in some cases. A summary of the outstanding morphological features of the urediniospores of the biologic forms studied is given below.

P. graminis tritici.—The urediniospores are quite constant in size, shape, and color. They are the longest of all the biologic forms of *P. graminis*, but in width they exceed only slightly those of *P. graminis avenae*. Their shape is elliptic to ovoid, color light cadmium-yellow.

P. graminis tritici-compacti.—The urediniospores are very similar to those of *P. graminis tritici*, but are slightly shorter, and consequently are inclined to be ellipsoid and oval. In color they are somewhat duller.

P. graminis secalis.—The spores are uniform in size, color, and shape. The color is dull, ashy yellowish to grayish; in length they are somewhat shorter than those of *P. graminis* on oats, width approaching that of spores of *P. graminis phleipratensis;* in shape cylindric-elliptic.

P. graminis avenae.—The size and shape of the urediniospores are very variable. The shape ranges from ellipsoid to ovoid to pyriform to subphobose, even when grown on its type host, *Avena sativa*. Their color is similar to that of spores of *P. graminis tritici*.

P. graminis phleipratensis.—The spore shape is predominantly pyriform; they are very short and fairly uniform; their color is even duller and more grayish than that of spores of *P. graminis secalis*.

P. graminis agrostis.—The spores are remarkably constant in size, but are smaller than those of any other form. In color and shape they resemble spores of *P. graminis phleipratensis*, but possibly are not quite so pyriform.

The spore dimensions for the above biologic forms are given in Table III, in order to facilitate ready comparison. The "size limits" in this table show the extreme variations of all of the urediniospore dimensions

of a given form studied. The "mode averages" were obtained by finding the arithmetic mean of all modes of a given biologic form cultured on various congenial hosts.

TABLE III.—*Comparative sizes of urediniospores of biologic forms of Puccinia graminis*

Biologic form.	Size limits	Mode averages
	μ	μ
P. graminis tritici	23. 04–41. 92 × 15. 04–24. 96	32. 36 × 19. 82
P. graminis tritici compacti	23. 68–40. 00 × 14. 40–25. 28	31. 72 × 19. 48
P. graminis secalis	17. 92–38. 72 × 13. 44–21. 44	27. 14 × 17. 20
P. graminis avenae	19. 20–37. 12 × 13. 76–25. 60	28. 48 × 19. 46
P. graminis phleipratensis	16. 00–32. 00 × 11. 84–21. 12	23. 04 × 17. 24
P. graminis agrostis	15. 04–31. 08 × 12. 16–20. 48	22. 48 × 15. 95

Table III shows distinctly the considerable variation in the size of spores of the different biologic forms. The uredinospores of *P. graminis tritici* are the largest of all, while those of *P. graminis tritici-compacti* are less than 1μ shorter and only a fraction of a micron narrower. The other forms vary more perceptibly. The spores of *P. graminis secalis* approach those of *P. graminis avenae* in length, the latter resembling those of *P. graminis tritici* in width. The spores of *P. graminis phleipratensis* are similar in width to those of *P. graminis secalis*, but considerably shorter; while *P. graminis agrostis* has smaller uredinospores than any other biologic form of *P. graminis* studied.

Relative to shape, the six biologic forms discussed in this paper could be classified in two principal groups; the ellipsoid-cylindric group, consisting of *P. graminis tritici*, *P. graminis tritici-compacti*, and *P. graminis secalis;* and the ovoid-subglobose group, including *P. graminis avenae*, *P. graminis phleipratensis*, and *P. graminis agrostis*. Stakman and Piemeisel (*14*) made an identical classification of these forms on the basis of their parasitism.

It is interesting to note that the morphological differences between the individual biologic forms of *Puccinia graminis* are fully as great and distinct as those between many generally recognized species of fungi. Because of similar morphological variation in certain biologic forms of *Erysiphe graminis*, expressed by distinctive characteristics in the color of the conidia and in some cases also in their size, Salmon (*9*) concluded that those forms were "incipient morphological species." The same may be true of the biologic forms of *Puccinia graminis*.

INFLUENCE OF HOST

If biologic forms of *Puccinia graminis* are incipient species, they are probably evolving gradually. If the change is sudden and accidental, finding the evidence may be merely a matter of chance. If, however,

the change is a gradual one, it is reasonable to hope that some evidence of this change may be obtained by the methods used in the present work.

Two lines of work were pursued: (1) Attempts were made to develop a number of morphological strains of a given biologic form by culturing it for fairly long periods of time on several different hosts, and (2) attempts were made to unify spore sizes of different biologic forms by growing them on the same hosts. For instance, *P. graminis tritici* develops well on common wheat, barley, and on various species of Agropyron, Hordeum, and Elymus. The writers tried to ascertain whether these hosts exerted an appreciable effect on the rust when it had been confined to them for considerable periods of time. Again the *avenalis*, *tritici*, and *tritici-compacti* forms grow about equally well on barley. Theoretically, therefore, it could be assumed that they ought to become morphologically similar if grown on barley long enough. In fact, all of the biologic forms discussed in this paper develop at least weakly on barley. It could be assumed that if they could all be grown on barley long enough, they would eventually become similar morphologically. The results of the effect of hosts are given on Tables IV to XII.

KEY TO TABLES IV TO XII

In Tables IV to XII the host from which the rust was originally cultured is given in the second column. Intermediate hosts refer to the hosts on which the rust had been grown up to the time the plant was inoculated. The term "intermediate host" is not used here in the sense of bridging. W, O, B, and R refer to wheat, oats, barley, and rye, respectively. Other symbols are explained when used. The number of "uredinio spore generations" (successive transfers) on the host is indicated by the figure immediately following the symbol for that host. Thus, $R_2B_4R_1W_2B_5$ indicates that the rust was transferred to rye twice, then to barley four times, followed by one transfer to rye, two to wheat, and five to barley. The degree of infection is self-explanatory. The result of inoculation is given in the usual manner in the form of a fraction, the denominator showing the number of plants inoculated and the numerator the number which became infected.

ATTEMPTS TO DEVELOP MORPHOLOGIC STRAINS OF BIOLOGIC FORMS BY CULTURING ON DIFFERENT HOSTS

To determine whether or not a given biologic form of *P. graminis* has a tendency to split up into a number of different morphological strains on account of confinement for fairly long periods of time to a number of different hosts, a series of experiments was conducted with the six biologic forms indicated above. The host plants employed were very frequently of distant taxonomic relationship, but, unless they were equally congenial to the parasitic attack of the fungus, their effect was not considered when the final conclusions were drawn. The result of this phase of the work, which extended over a period of two years, is given in Tables IV to IX.

Many inoculations were made with *P. graminis tritici* on wheat, barley, rye, and *Hordeum jubatum* (Table IV). All except rye are very susceptible. On congenial hosts the spores remained quite constant in shape, size, and color, irrespective of their origin and subsequent history. On rye, however, an uncongenial host, both the uredinia and the urediniospores became appreciably smaller, especially in length.

These results are not in accordance with those of Freeman and Johnson (*3*, *p.* 28), who say:

> The host-plant exercises a strong influence, not only on the physiological and biological relationship, but in some cases even on the morphology of the uredospores.

The difference in results might possibly be explained by supposing that Freeman and Johnson worked with a mixed strain, or that they did not measure enough spores. Their rust, however, may actually have changed. It will be readily seen from Table IV that the writers were not able to change the dimensions more than about 1 μ, which is within the range of experimental error.

The color of the urediniospores of *P. graminis tritici* is pale cadmium-yellow; their shape predominantly elliptic to ovoid; size limits 23 to 42 by 15 to 25 μ, and average modes 32.36 by 19.82 μ.

Results obtained by Stakman and Piemeisel (*14*) showed that the biologic form of rust, *P. graminis tritici-compacti*, discovered west of the Rocky Mountains on several different grasses and on club wheat, varied parasitically from *P. graminis tritici*, found east of the Rockies. Many common *aestivum* wheats, such as Haynes Bluestem and Fife, are resistant to this biologic form, while barley is fairly tolerant and the club wheats inoculated and Pacific Bluestem are very susceptible. Spore measurements of over a dozen strains (Table V) indicate that, whereas the spore sizes on the susceptible hosts vary but little (less than 1 μ) from those of *P. graminis tritici*, yet they are on the average nearly 2 μ shorter on the tolerant hosts and almost 4 μ shorter on the resistant ones. The width of the spores does not seem to be influenced by the host.

Identical results were obtained with *P. graminis tritici-compacti*, found in the summer of 1917 in Louisiana and Alabama, on several soft wheats. The constancy of size is remarkable, and, like the western strain, the southern strain, too, exhibits a special affinity for club wheats, while the most of the hard wheats are resistant.

The color of the urediniospores of *P. graminis tritici-compacti* is practically the same as that of *P. graminis tritici*. The spores are slightly shorter, and ovoid to ellipsoid in shape.

TABLE IV.—*Effect of various hosts on the morphology of urediniospores of Puccinia graminis tritici*

[H_j=Hordeum jubatum. x=Long time association with host; number of urediniospore generations indefinite]

Experiment No.	Original host	Intermediate host	Plant inoculated	Degree of infection.	Result.	Spore dimensions	
						Size limits	Modes
						μ	μ
1	*Hordeum jubatum*	None	*Triticum aestivum*	Heavy	16 16	23. 04–40. 32×15. 68–24. 96	31. 68×19. 84
2	do	do	do	do	31 33	23. 04–40. 32×15. 04–23. 04	32. 32×19. 52
3	do	W_4	do	do	33 26	23. 04–38. 08×16. 00–22. 40	32. 00×19. 52
4	do	W_2	do	do	27 19	25. 60–40. 96×16. 96–23. 36	32. 96×20. 16
5	*Agropyron smithii*	$W_2B_{14}Hj_1$	do	do	19 13	25. 60–39. 68×16. 96–22. 72	32. 64×19. 84
6	*Triticum aestivum*	W_x	do	do	14	25. 60–40. 32×16. 32–23. 36	32. 96×19. 84
7	*Hordeum jubatum*	W_2	*Hordeum vulgare*	do	32 32	24. 92–40. 00×16. 00–24. 96	32. 00×20. 48
8	do	W_2B_8	do	Moderate	23 33	26. 24–38. 72×15. 36–23. 04	31. 36×19. 20
9	do	W_2B_8	do	Heavy	31	25. 60–38. 72×16. 32–22. 72	32. 00×19. 52
10	do	W_8	do	do	16 18	24. 00–40. 64×16. 64–23. 68	32. 32×20. 16
11	do	W_8B_2	do	do	25 25	24. 32–40. 96×16. 32–23. 36	32. 64×19. 84
12	do	B_{35}	do	do	27 27	25. 60–38. 72×16. 64–23. 04	32. 32×19. 84
13	do	$R_2B_4R_1W_2B_6$	do	do	28 28	25. 28–40. 64×16. 64–23. 04	32. 96×19. 84
14	*Agropyron tenerum*	B_{14}	do	do	26 26	25. 60–38. 40×15. 04–23. 36	32. 00×19. 20

15	*A. smithii*	W_2B_{14}	do	do	$\frac{30}{30}$	23. 68–41. 92×17. 28–22. 72	32. 64×19. 84
16	*Hordeum jubatum*	W_6	*Hordeum jubatum*	do	$\frac{23}{26}$	24. 96–40. 32×17. 28–23. 36	32. 64×20. 16
17	do	W_6H_2	do	Moderate	$\frac{19}{19}$	24. 64–40. 64×17. 60–23. 04	32. 64×20. 16
18	*Agropyron smithii*	$W_2B_{14}H_2$	*Secale cereale*	Weak	$\frac{2}{14}$	23. 04–36. 48×16. 96–23. 04	30. 08×19. 84

TABLE V.—*Effect of various hosts on the morphology of urediniospores of Puccinia graminis tritici-compacti*

[C=Club wheat. M=Marquis wheat]

Experiment No.	Original host	Place of collection	Intermediate hosts.	Plant inoculated	Degree of infection.	Result	Spore dimensions	
							Size limits	Modes
							μ	μ
1	*Triticum compactum*	Pullman, Wash.	B_3C_1	Pacific Bluestem wheat.	Heavy	10 11	26.24–37.44×10.96–22.08	31.68×19.52
2	do	do	B_3C_1	*Triticum compactum.*	do	26 30	25.60–38.08×14.40–24.00	32.00×19.20
3	*Elymus condensatus*	do	W_1C_3	do	do	28 31	25.28–37.76×14.40–25.28	31.68×19.52
4	do	Ritzville, Wash	W_2	do	do	57 58	24.96–37.76×16.64–22.72	31.68×19.84
5	*Hordeum vulgare*	Corvallis, Ore	C_2	do	do	9 10	24.64–37.76×16.00–22.40	31.68×19.20
6	*Secale cereale*	Baton Rouge, La	B_4C_2	do	Moderate	3 5	24.96–37.76×15.68–23.36	31.68×19.52
7	*Triticum aestivum*	Brundidge, Ala	$B_2W_1C_3$	do	Heavy	10 18	24.00–38.72×16.64–22.40	31.36×19.52
8	*Elymus glaucus*	Ellensburg, Wash	$W_1B_2M_1B_1$	*Hordeum vulgare*	do	24 24	24.00–36.16×16.96–21.76	30.08×19.52
9	*Hordeum jubatum*	Pullman, Wash	None	do	do	31 31	24.00–37.76×16.00–22.72	30.72×19.52
10	*Elymus glaucus*	Ellensburg, Wash	do	*Triticum aestivum*	do	16 17	21.44–36.48×15.68–22.08	26.56×18.88
11	do	do	W_1B_1	do	do	30 36	19.84–34.56×17.28–21.76	27.84×19.52
12	*Triticum compactum*	do	$B_2W_1C_1$	do	do	13 15	22.40–33.92×16.00–23.36	28.16×19.52
13	*Triticum aestivum*	Brundidge, Ala	$B_2W_1C_2$	do	do	10 16	21.44–33.92×16.96–22.72	28.16×19.52

14	*Triticum compactum*	Pullman, Wash.	B_2C_1	Turkey (Minn.,820)	do	$\frac{9}{10}$	23.04–33.60×17.28–22.72	28.48×19.84
15	do	do	B_3C_1	*Triticum dicoccum*	do	$\frac{7}{11}$	23.36–34.24×16.32–23.04	28.80×19.52
16	*Elymus glaucus*	do	None	*Secale cereale*	do	$\frac{4}{27}$	21.12–36.16×16.32–22.40	28.16×19.20

TABLE VI.—*Effect of various hosts on the morphology of urediniospores of Puccinia graminis secalis*

[As=*Agropyron repens*, At=*A. tenerum*; Ec=*Elymus canadensis*; Er=*E. robustus*; Ev=*E. virginicus*; Hj=*Hordeum jubatum*, x=Long-time association with host, number of urediniospore generations indefinite]

Experiment No.	Original host.	Intermediate hosts.	Plant inoculated.	Degree of infection.	Result.	Spore dimensions.	
						Size limits.	Modes.
						μ	μ
1	*Agropyron repens*	None	*Secale cereale*	Heavy	21 23	20. 16–38. 72×13. 44–21. 44	28. 10×17. 28
2	do	R_1	do	Moderate	26 42	17. 92–32. 00×13. 76–20. 16	25. 00×17. 28
3	do	R_7	do	Heavy	27 36	21. 12–33. 28×14. 72–20. 16	26. 88×17. 28
4	*Hordeum jubatum*	None	do	do	24 27	19. 84–36. 48×14. 08–20. 80	27. 20×16. 90
5	do	R_4	do	Weak	32 28	21. 12–34. 24×13. 44–21. 12	26. 56×17. 28
6	do	R_7	do	Heavy	27 29	19. 20–34. 88×14. 40–20. 16	27. 20×17. 28
7	*Secale cereale*	R_x	do	Moderate	24 25	20. 48–33. 92×14. 08–19. 52	27. 52×16. 64
8	*Agropyron repens*	None	*Horceum vulgare*	Weak	13 33	19. 20–34. 88×15. 04–19. 84	26. 24×17. 28
9	do	R_5	do	Moderate	18 20	19. 52–35. 20×14. 72–19. 84	27. 52×17. 28
10	do	R_5B_2	do	do	26 29	23. 04–33. 28×13. 76–20. 48	28. 16×16. 96
11	*Hordeum jubatum*	R_5	do	Weak	16 18	19. 20–34. 88×14. 40–20. 16	27. 20×17. 28
12	do	R_5B_2	do	Moderate	25 25	21. 76–33. 92×14. 72–19. 52	27. 52×16. 96
13	*Agropyron repens*	B_{13}	do	do	12 12	20. 48–37. 44×15. 36–19. 20	28. 80×17. 28

14	*Agropyron smithii*	$R_2B_4R_2B_3$	do	do	15 16	21. 44–35. 52×14. 40–19. 84	28. 16×16. 96
15	*Hordeum jubatum*	$R_1W_1R_6B_5$	do	do	16 21	22. 72–32. 96×14. 40–19. 84	27. 84×17. 28
16	*Hystrix patula*	$R_2B_4R_2B_6$	do	do	13 17	21. 76–32. 32×15. 04–20. 16	27. 20×17. 60
17	do	$R_2B_4R_2B_3Ar_1B_1$ $Ev_1At_4B_5$	do	Weak	38 43	21. 44–32. 32×15. 04–20. 16	27. 20×17. 60
18	do	$R_2B_4R_2B_3Ar_1B_1$ $Ev_1At_4B_7$	do	Moderate	18 25	22. 08–33. 60×13. 44–21. 44	27. 20×17. 60
19	*Hordeum jubatum*	None	*Hordeum jubatum*	do	9 11	20. 48–35. 52×13. 76–20. 16	26. 56×16. 96
20	do	R_5	do	do	9 18	18. 88–35. 20×14. 08–20. 48	27. 20×17. 28
21	do	R_5Hj_2	do	do	22 22	20. 48–33. 60×14. 08–20. 16	27. 52×16. 96
22	do	Hj_4	do	do	14 18	19. 84–32. 00×13. 44–21. 12	25. 92×16. 96
23	*Agropyron repens*	None	*Agropyron repens*	Weak	3 13	17. 92–32. 96×14. 08–20. 48	25. 92×17. 28
24	do	Ar_4	do	Moderate	23 29	19. 52–33. 92×15. 04–20. 16	25. 60×17. 60
25	do	Ar_7	do	Heavy	23 23	19. 52–35. 52×14. 40–20. 16	27. 52×17. 28
26	do	$B_1R_1B_7Er_{12}Ec_5Ev_5$	*Elymus virginicus*	do	67 67	21. 76–33. 28×15. 04–20. 16	27. 52×17. 60
27	*Hystrix patula*	$R_2B_4R_2B_3Ar_1B_1Ev_1$	*Hystrix patula*	do	22 22	21. 12–32. 96×15. 04–20. 48	27. 20×17. 60
28	do	$R_2B_4R_2B_3Ar_1B_1$ Ev_1At_{10}	*Agropyron tenerum*	Weak	60 63	18. 88–34. 24×14. 72–20. 48	26. 88×17. 60

TABLE VII.—*Effect of various hosts on the morphology of urediniospores of Puccinia graminis avenae*

[Pp=*Phleum pratense*; Dg=*Dactylis glomerata*; Bt=*Bromus tectorum*, X=Long-time association with host, number of urediniospore generations indefinite]

Experiment No.	Original host.	Intermediate hosts.	Plant inoculated.	Degree of infection.	Result	Spore dimensions	
						Size limits	Modes.
						μ	μ
1	*Dactylis glomerata*	None	*Avena sativa*	Heavy	21/21	20. 80–37. 12×16. 00–25. 60	27. 84×19. 84
2	do	do	do	Moderate	4/35	19. 20–35. 20×13. 76–23. 04	26. 88×18. 56
3	do	O_5	do	Heavy	19/19	22. 08–34. 88×16. 00–22. 40	28. 48×19. 20
4	do	O_8	do	do	25/25	21. 12–36. 48×16. 32–23. 04	29. 12×19. 52
5	do	O_{22}	do	do	23/23	19. 52–36. 48×16. 00–23. 36	28. 48×20. 16
6	do	$O_{43}Pp_1$	do	1 uredinium.	1/5	22. 72–36. 16×16. 00–22. 72	29. 44×19. 52
7	*Avena sativa*	O_x	do	Heavy	22/23	23. 04–35. 20×16. 96–22. 08	29. 12×19. 52
8	*Dactylis glomerata*	None	*Dactylis glomerata*	do	18/18	19. 20–32. 96×16. 00–24. 00	25. 92×19. 84
9	do	do	do	do	11/15	18. 56–35. 20×15. 36–23. 36	26. 24×19. 52
10	do	O_6	do	do	11/12	19. 20–32. 00×16. 32–22. 40	25. 60×19. 52
11	do	O_6Dg_2	do	do	19/20	20. 16–31. 68×16. 64–22. 08	25. 92×19. 52
12	do	O_6	*Bromus tectorum*	Weak	10/10	18. 56–29. 12×16. 64–21. 12	23. 68×18. 88
13	do	O_6–Bt_2	do	do	5/12	19. 20–28. 80×16. 32–21. 12	23. 68×18. 88

14	....do.....	O_{31}.....	*Phleum pratense*.....	...do.....	$\frac{5}{50}$	20. 16–32. 64× (*a*).....	25. 60× (*a*)
15	....do.....	O_{33}.....	*Hordeum vulgare*.....	...do.....	$\frac{3}{15}$	20. 80–32. 96× (*a*).....	25. 60× (*a*)
16	....do.....	O_{36}.....	*Alopecurus pratensis*	Heavy.....	$\frac{60}{60}$	19. 84–33. 60×16. 96–22. 72..	26. 56×19. 84
17	....do.....	O_{34}.....	*Lolium temulentum*..	Moderate..	$\frac{4}{20}$	19. 52–33. 60×16. 64–22. 40..	26. 56×19. 52
18	....do.....	O_{32}.....	*Hordeum pusillum*...	Weak.....	$\frac{4}{28}$	19. 20–30. 40×17. 28–21. 76..	24. 32×19. 52

[a] Widths of the urediniospores were not measured.

TABLE VIII.—*Effect of various hosts on the morphology of urediniospores of Puccinia graminis phleipratensis*

[Dg=*Dactylis glomerata*; Pp=*Phleum pratense*; x=Long association with host; number of urediniospore generations indefinite]

Experiment No.	Original host.	Intermediate hosts.	Plant inoculated.	Degree of infection.	Result.	Spore dimensions	
						Size limits.	Modes.
1	*Phleum pratense*	None	*Phleum pratense*	Heavy	25 25	17. 92–29. 44×13. 12–21. 12	23. 68×17. 28
2	do	Pp_5	do	Moderate	18 24	16. 00–28. 80×13. 44–20. 16	21. 70×16. 96
3	do	Pp_8	do	Heavy	23 33	16. 32–29. 76×14. 40–19. 84	23. 04×17. 28
4	*Dactylis glomerata*	None	do	Weak	4 35	10. 64–28. 80×12. 80–18. 88	22. 08×16. 96
5	do	Pp_5	do	Heavy	13 13	16. 32–29. 12×13. 76–19. 84	22. 08×17. 28
6	do	Pp_8	do	do	20 21	18. 24–28. 48×14. 40–19. 20	23. 04×16. 96
7	*Phleum pratense*	Pp_x	do	do	18 18	17. 60–29. 44×13. 12–20. 48	24. 00×16. 96
8	do	None	*Dactylis glomerata*	Moderate	22 22	16. 64–29. 12×13. 76–20. 80	22. 08×17. 28
9	do	Pp_6	do	do	4 8	16. 32–28. 48×14. 40–20. 16	22. 40×17. 28
10	do	Pp_6Dg_2	do	do	18 20	19. 20–28. 48×14. 08–19. 84	24. 00×16. 96
11	*Dactylis glomerata*	Pp_6	do	do	9 12	16. 96–29. 44×13. 76–20. 48	23. 36×17. 28
12	do	Pp_6Dg_2	do	do	17 19	18. 56–28. 80×13. 44–19. 84	23. 68×16. 64

13	*Phleum pratense*	None	*Avena sativa*	Weak	14 53	17. 60–30. 08×14. 72–20. 48	22. 72×17. 60
14	do	P_{P_1}	do	do	6 12	16. 96–32. 00×13. 76–20. 48	23. 68×17. 28
15	do	P_{P_8}	do	do	8 21	17. 92–28. 16×15. 04–20. 80	23. 04×17. 92
16	*Dactylis glomerata*	P_{P_9}	do	do	2 13	17. 60–31. 36×11. 84–21. 12	23. 68×17. 28
17	do	$P_{P_9O_4}$	do	do	1 8	18. 56–28. 48×14. 72–20. 48	23. 36×17. 92
18	*Phleum pratense*	B_2	*Hordeum vulgare*	do	1 1	14. 08–27. 20×11. 20–17. 60	21. 76×14. 08
19	do	B_2	do	do	1 9	14. 08–25. 92×11. 52–17. 28	20. 80×14. 40

A rather distinct and consistent specificity in shape and color was exhibited by the rye rust urediniospores regardless of the host on which they developed, length of time confined to it, or of origin and subsequent history. As seen from Table VI, the spore modes average more than 5 μ shorter and about 2.5 μ narrower than those of the spores of *P. graminis tritici;* the shape is cylindric-elliptic, and the color dull, ashy-yellowish to grayish. The size of the spores is perceptibly affected by cultural conditions, but not by the host plants. Under the same circumstances the spores were in each case distinctly and consistently smaller than those of *P. graminis tritici.* During the cloudy and cold weather of midwinter and the excessive heat of the summer months the spores grew only to their minimum size and again attained their maximum size in spring and fall.

P. graminis avenae (Table VII) was thought to be an especially interesting form for study in the attempt to determine the amount of variation which could be induced by growing it on different congenial hosts, because it appeared from inoculation results that this biologic form might possibly be considered as plastic. In contrast to *P. graminis tritici, P. graminis tritici-compacti,* and *P. graminis secalis,* the urediniospores of which are, as a rule, of a definite specific size and shape when grown on a congenial host, those of *P. graminis avenae* are very variable even when parasitizing oats. They may be ellipsoid, ovoid, pyriform, or subglobose in shape. In size they are somewhat longer than those of *P. graminis secalis,* and in width approach those of *P. graminis tritici.* The spore color is bright cadmium-yellow.

On *Dactylis glomerata* the spores show a tendency to shorten, the width remaining practically the same as on oats. On *Bromus tectorum,* which is only a tolerant host, the spores decreased both in length and width, becoming nearly globose. The presence of equatorial germ pores, however, showed clearly that the rust was *P. graminis.* Both barley and timothy are very uncongenial hosts for *P. graminis avenae* and both uredinia and spores are very small (*13*). This is similar to the behavior of *P. graminis tritici* on resistant varieties of wheat on which Stakman (*11, p. 31*) found the spores to be smaller than on the susceptible wheat varieties. Similar results were obtained by the writers with *P. graminis tritici-compacti* (see Table V).

It will thus be seen that the size and shape of *P. graminis avenae* are quite easily influenced by the host on which they grow; the color, however, remains constant. The size of uredinia is directly proportional to the size of the spores, and vice versa. This morphological variation is interesting because the rust is also versatile parasitically.

TABLE IX.—*Results showing the effect of various hosts on the morphology of urediniospores of Puccinia graminis agrostis*

[Aa=*Agrostis alba*]

Experiment No.	Original host.	Place of collection.	Intermediate host.	Plant inoculated.	Degree of infection.	Result.	Spore dimensions.	
							Size limits.	Modes.
							μ	μ
1	*Agrostis alba*.	Crookston, Minn.	Aa_1	*Agrostis alba*	Heavy	50/50	18.88–26.56×13.76–18.24	22.72×16.00
2	do	do	Aa_2	do	do	100%	17.60–28.48×14.08–18.88	22.72×16.32
3	do	do	Aa_2	*Alopecurus pratensis*	Moderate to heavy.	20/30	16.32–28.80×13.12–18.24	22.40×15.68
4	do	do	Aa_2	*Holcus lanatus*	Moderate	35/45	17.92–27.20×13.44–17.92	22.40×15.68
5	do		None.	*Hordeum vulgare*	Weak	7/26	15.04–30.08×13.12–18.56	22.40×16.00
6	do		do.	*Secale cereale*	do	2/17	18.24–25.92×13.44–17.28	21.44×16.00
7	do	Long Beach, Cal.	do.	*Avena sativa*	do	4/36	15.68–28.48×12.16–20.16	22.08×16.00

TABLE X.—*Results of using Hordeum vulgare as a common host to unify the size of uredinispores of Puccinia graminis tritici and Puccinia graminis secalis*

[Hj=*Hordeum jubatum*]

Experiment No.	Biologic form.	Original host.	Subsequent hosts.	Degree of infection.	Result.	Spore dimensions	
						Size limits.	Modes.
						μ	μ
1	*P. graminis tritici*	*Hordeum jubatum*	W_2B_1	Heavy	32	25. 92–40. 00×16. 00–24. 96	32. 00×20. 48
2	do	do	W_2B_6	Moderate	31	20. 24–38. 72×15. 36–23. 04	31. 36×19. 20
3	do	do	W_2B_9	do	31	25. 60–38. 72×16. 32–22. 72	32. 00×19. 52
4	do	do	Hj_2B_1	do	16	24. 00–40. 64×16. 64–23. 68	32. 32×20. 16
5	do	do	Hj_2B_4	do	18	24. 32–40. 96×16. 32–23. 36	32. 64×19. 84
6	do	do	B_{16}	Heavy	25	25. 60–38. 72×16. 64–23. 04	32. 32×19. 84
7	do	do	$R_2B_4R_1W_2B_6$	do	27	25. 28–40. 64×16. 64–23. 04	32. 96×19. 84
8	do	*Agropyron tenerum*	B_{15}	do	28	25. 60–38. 40×15. 04–23. 36	32. 00×19. 20
9	do	*Agropyron smithii*	W_2B_{15}	do	26	23. 68–41. 92×17. 28–22. 72	32. 64×19. 84
10	*P. graminis secalis*	*Agropyron repens*	None	Weak	30	19. 20–34. 88×15. 04–19. 84	26. 24×17. 28
11	do	do	R_3B_1	Moderate	13	19. 52–35. 20×14. 72–19. 84	27. 52×17. 28
12	do	do	R_3B_2	do	18	23. 04–33. 28×13. 76–20. 48	28. 16×16. 96
13	do	do	B_{14}	do	26	20. 48–37. 44×15. 36–19. 20	28. 80×17. 28
					29		
					12		
					12		

14	....do....	*Hordeum jubatum*....	R_3B_1....	Weak....	16 18	19. 20–34. 88×14. 40–20. 16	27. 20×17. 28
15	....do....	....do....	R_3B_2....	Moderate....	25 25	21. 76–33. 92×14. 72–19. 52	27. 52×16. 96
16	....do....	....do....	$R_1W_1R_6B_7$....	....do....	16 21	22. 72–32. 96×14. 40–19. 84	27. 84×17. 28
17	....do....	*Hystrix patula*....	$R_2B_4R_2B_7$....	....do....	13 17	21. 76–32. 32×15. 04–20. 16	27. 20×17. 60
18	....do....	*Agropyron smithii*....	$R_3B_4R_2B_4$....	....do....	15 16	21. 44–35. 52×14. 40–19. 84	28. 16×16. 96

Johnson (*6, p. 8*) gives the urediniospore dimensions of timothy-rust as 18 to 27 μ in length and 15 to 19 μ in width. Stakman and Jensen (*12, p. 214*) found that on timothy the spores ranged from 17 to 31 μ in length and from 14.5 to 23 μ in width, the modes falling at about 26 and 18 μ. On barley they found them to be smaller than those produced on any other host, ranging from 18.5 to 28.3 μ in length and from 13 to 20 μ in width, with modes at about 23 and 17 μ. In the present work it was found that all the modes, except those of spores cultured on barley, fluctuated about those of a strain of *P. graminis phleipratensis*, which had been confined to timothy for more than a year. The modes fluctuated about 24 and 17 μ, varying perceptibly with the existing climatic and edaphic conditions.

That barley is not a congenial host for timothy-rust is shown by the slight virulence of infection (Table VIII), very small uredinia, and considerably decreased size of the urediniospores, with average mode of 21.28 by 14.24 μ. The results of inoculation with *P. graminis phleipratensis*, obtained from two different sources—timothy (*Phleum pratense*) and orchard-grass (*Dactylis glomerata*)—and grown on three different hosts (timothy, orchard-grass, and oats), show (Table VIII) that the urediniospores retain their characteristic size, except for small negligible variations, whether parasitizing very congenial or merely tolerant hosts. The spore shape is predominantly pyriform, and the color is dull, dirty yellow to grayish.

The infection capabilities of *P. graminis agrostis* are similar to those of *P. graminis phleipratensis* and *P. graminis avenae*. The urediniospores of this rust are the smallest of all the biologic forms of *P. graminis*, especially in width. In shape and color they resemble very closely timothy-rust spores although not quite so dominantly pyriform. The spore dimensions are given in Table IX.

The results given in Table IX show clearly that the influence of host on the size of the spores was negligible.

Attempts to Unify Spore Sizes of Different Biologic Forms by Culturing Them on the Same Host

P. graminis tritici has a number of hosts in common with *P. graminis secalis*. *P. graminis avenae* and *P. graminis phleipratensis* also have several hosts in common. The work was directed toward an attempt to determine whether the spore morphology of these biologic forms could be made identical by the use of common hosts. Table X gives the results of using *Hordeum vulgare* as a common host to unify *P. graminis tritici* and *P. graminis secalis*, and in Table XI are given the results of using *Avena sativa* as a common host for *P. graminis avenae* and *P. graminis phleipratensis*. A condensed tabulated summary of these results is given in Table XII.

TABLE XI.—*Results of using Avena sativa as a common host to unify the size of urediniospores of Puccinia graminis avenae and Puccinia graminis phleipratensis*

[*Pp*=*Phleum pratense*]

Experiment No.	Biologic form.	Original host.	Subsequent hosts.[1]	Degree of infection.	Result.	Spore dimensions.	
						Size limits.	Modes.
						μ	μ
1	*P. graminis avenae*	*Dactylis glomerata*	O_1	Heavy	21/21	20.80–37.12×16.00–25.60	27.84×19.84
2	do	do	O_1	Moderate	4/35	19.20–35.20×13.76–23.04	26.88×18.56
3	do	do	O_6	Heavy	19/19	22.08–34.88×16.00–22.40	28.48×19.20
4	do	do	O_8	do	25/25	21.12–36.48×16.32–23.04	29.12×19.52
5	do	do	O_{22}	do	32/32	19.52–36.48×16.00–23.36	28.48×20.16
6	*P. graminis phleipratensis*	*Phleum pratense*	O_1	Weak	14/53	17.60–30.08×14.72–20.48	22.72×17.60
7	do	do	Pp_1O_1	do	6/12	16.96–32.00×13.76–20.48	23.68×17.28
8	do	do	Pp_8O_1	do	8/21	17.92–28.16×15.04–20.80	23.04×17.92
9	do	*Dactylis glomerata*	Pp_6O_1	do	2/13	17.60–31.36×11.84–21.12	23.68×17.28
10	do	do	Pp_6O_3	do	1/8	17.60–28.48×14.72–20.48	23.36×17.92

TABLE XII.—*Comparative sizes of urediniospores of Puccinia graminis as affected by common hosts. Summary of Tables X and XI*

Biologic form	Host.	Size limits.	Mode averages.
		μ	μ
P. graminis tritici	*Hordeum vulgare*	24. 00–41. 92×15. 04–24. 96	32. 25×19. 77
P. graminis secalis	....do	19. 20–37. 44×13. 76–20. 48	27. 63×17. 21
P. graminis avenæ	*Avena sativa*	19. 20–37. 12×13. 76–25. 60	28. 16×19. 46
P. graminis phlebratensis.	....do	16. 96–32. 00×11. 84–21. 12	23. 29×17. 60

It is evident from Table XII that barley can not change or unify the wheat and rye forms; neither can oats do so with the oats and timothy forms. This substantiates the writers' results on the constancy and stability of the biologic forms of *P. graminis* in general. Only uncongenial hosts appear to have the property of changing the morphology of urediniospores as expressed by size or shape. And even in such cases the urediniospores resume their original size and shape when grown again on congenial hosts.

EFFECT OF PHYSICAL FACTORS

The attempts to change the spore morphology by means of physical factors are given in Tables XIII to XVII. While some of the observations on the effect of these factors on the development of the rust are repetitions of those previously made by other investigators and while some of the others may seem perfectly obvious, they are made to show the correlation, if any exists, between the vigor of the fungus and the morphological characters of the spores. At the same time some of the results on the development of the rust under various conditions are valuable in themselves.

EFFECT OF TEMPERATURE

In the present experiment on temperature wheat seedlings were inoculated with fresh urediniospores of *P. graminis tritici* in the usual manner, given normal germination conditions, and then exposed to various temperatures. The high temperature was obtained by means of an electric heater put under a glass bell jar where the plants were kept continuously. For low temperature the plants under a bell jar, as in the above case, were kept either in an unheated greenhouse or outside, according to existing conditions. The temperatures were recorded by thermographs from which the records were then computed. A set of control plants was kept under normal greenhouse conditions (Table XIII).

TABLE XIII.—*Results showing the effect of temperature on the physiology and morphology of urediniospores of Puccinia graminis tritici on wheat*

Experiment No.	Temperature.			Degree of infection.	Result	Spore dimensions.	
	Daily mean.		Average for generation.			Size limits.	Modes.
	Maximum.	Minimum.					
	°F.	°F.	°F.			μ	μ
1	92.3	76.4	81.8	Moderate	13	21.04–36.48 × 16.1–24.15	29.44 × 19.84
2	103.5	71.1	79.5	do	21	24.64–40.00 × 16.42–24.96	29.44 × 20.16
3	78.0	61.0	69.7	Heavy	24	26.92–40.00 × 16.96–24.16	31.04 × 20.16
4	79.4	63.2	69.3	do	18	22.40–40.32 × 16.32–24.68	29.60 × 19.84
5	89.7	52.3	66.4	do	16	25.60–40.00 × 16.00–23.04	31.28 × 19.84
6	72.0	40.8	55.8	do	16	26.28–40.00 × 18.24–22.72	32.64 × 20.48
7	62.9	40.3	55.8	do	12	26.24–40.00 × 16.96–22.72	31.36 × 19.84

It was found that the most favorable temperature for shortening the incubation period, hastening the maturity and obtaining a vigorous infection, appeared to be between 66.5° and 70° F. Fromme's tabulation (*4*, *p.* 507–509) shows that this is in accordance with the results obtained by Wüthrich on *P. graminis* and by Ward on *P. dispersa*. This temperature is also the optimum for the production of the largest urediniospores. The reason the spores in No. 6, Table XIII, became so large is on account of the high maximum temperature.

At this temperature (66.5°–70° F.) rust flecks appeared in from five to seven days and uredinia developed within another day or two. At a higher temperature the development of the uredinia was retarded at the rate of one day for every 10 degrees of rise of temperature, but rust developed at as high temperatures as the host endured, although the size of the spores was considerably decreased. At low temperatures the development of the uredinia was retarded at the rate of one day for every 5 degrees of fall in temperature. Infection resulted at as low temperatures as the host could possibly stand. The spores were rather small, but the difference was not as great as in the case of high temperatures, with moderate temperature as a basis for comparison.

The uredinia produced under high temperatures were darker in color than those produced under moderate temperatures, while those produced at low temperatures were lighter than those produced at moderate temperatures. The color of the uredinia developed at high temperatures varied from Brussels-brown to argus-brown; at moderate temperature it varied from Sudan-brown or antique-brown to Brussels-brown, while at low temperature from amber-brown to Sanford's-brown.

EFFECT OF HUMIDITY

Plants for this experiment on humidity (Table XIV) were placed under two glass bell jars, under one of which there were exposed three beakers filled with water to secure a humid atmosphere; under the other bell jar three plates with calcium chlorid were placed to absorb the moisture in the air and that of transpiration by the plants. To prevent the evaporation of moisture from the soil in one case and the absorption of moisture by the soil in the other case, the surface of the pots was covered with a paraffin layer before they were placed under the jar. A third set of plants was kept as a control under normal greenhouse conditions. For the purpose of determining the relative humidity, hygrometers were employed for each set, and readings were taken daily; at the same time barometric readings in another end of the same building were being taken.

TABLE XIV.—*Results showing the effect of humidity on the physiology and morphology of uredinios pores of Puccinia graminis tritici on wheat*

Humidity						Spore dimensions.	
Experiment No.	Daily limits.		Total average.	Degree of infection.	Result	Size limits.	Modes.
	Maximum.	Minimum.					
	Per ct.	*Per ct.*	*Per ct.*			μ	μ
1	92.5	76.5	84.1	Moderate	22 23	23.36–40.32×16.64–23.04	32.96×19.84
2	67.5	47.0	60.4	Heavy	16 18	25.60–40.00×16.00–23.04	32.28×19.84
3	64.0	50.5	52.3	Weak	23 25	24.96–40.32×15.36–24.32	32.00×20.16
4	97.0	91.0	94.6	Moderate	5 6	24.96–40.32×15.68–24.96	32.32×20.80
5	83.5	73.0	78.5	do	18 15	22.40–40.32×16.32–23.68	32.00×19.84
6	66.5	39.5	62.2	do	7 7	22.72–39.36×15.68–24.64	30.40×19.84
7	95.0	90.5	93.4	Heavy	12 12	24.00–40.00×16.64–24.00	32.00×20.16
8	80.0	68.5	74.1	do	20 20	25.28–40.32×16.96–23.04	32.64×19.84
9	76.0	67.5	71.0	do	12 12	23.68–39.04×15.36–24.00	31.36×19.52

From the results shown in Table XIV, it would seem as though either excessively high or excessively low humidity causes a decrease in size of spores, but the difference, however, is neither very pronounced nor consistent. This can be explained by the fact that the inoculated plants were placed under the restricted conditions only subsequent to the usual confinement of 48 hours in the moist chambers. The germination period would thus appear to constitute the critical period, the difference in humidity thereafter being apparently of lesser importance.

The uredinia were generally larger on the control plants and smaller on those grown in the dry air. The color of the uredinia grown in humid atmosphere was commonly antique-brown or amber-brown, those produced in the arid atmosphere were amber to argus-brown, while on the control plants they were Sudan to Brussels-brown; in other words, they were darkest in the dry air and lightest in the moist air.

Effect of Soil Moisture

In this experiment on soil moisture three series of plants were employed, one of which was very heavily watered, the second moderately, and the third received only enough water to prevent the plants from wilting. Otherwise, the usual methods of inoculation, germination, and incubation were used. The water content of the soil was determined on the basis of the oven-dried method.

The plants in the wet soil were more severely attacked, and the urediniospores developed on them were larger than those in either the control or the dry series. The series that suffered from drouth produced the smallest spores. There was no apparent difference in the color of the uredinia or spores. Table XV gives the detailed results.

TABLE XV.—*Results showing the effect of soil moisture on the physiology and morphology of uredinisopores of Puccinia graminis tritici on wheat*

Experiment No.	Soil moisture.		Degree of infection.	Result.	Spore dimensions.	
	Water applied.	Water content.			Size limits.	Modes.
	Cc.	*Per cent.*			μ	μ
1	900	32.14	Heavy	20/20	22.72-43.84×16.96-22.40	33.28×19.84
2	850	31.65	do	16/16	26.24-46.96×17.60-22.72	33.28×20.16
3	400	17.12	do	20/20	25.28-46.32×16.96-23.04	32.64×19.84
4	250	16.28	do	24/24	26.56-49.60×16.96-23.36	32.64×20.16
5	150	9.24	Moderate	18/18	24.96-48.72×16.32-23.36	32.32×19.84
6	100	5.35	do	20/20	22.40-48.64×16.64-21.76	30.40×19.20

Effect of Illumination

In testing the effect of illumination two series of plants were employed, one of which was kept beneath a double-layer, muslin cage, while the other one was exposed to the direct sunlight in the greenhouse (Table XVI). As the experiment was conducted during the winter months the light was at no time exceptionally bright. The cultural conditions, except for the variation in light intensity, were maintained the same for both series. The light readings were taken daily, sometimes two and three times a day, with the Clements photometer charged with a

printing-out photographic paper. The percentage of intensity was determined by means of a standard print made at noon of a bright sunny day in the fall of 1915.

TABLE XVI.—*Results showing the effect of illumination on the physiology and morphology of urediniospores of Puccinia graminis tritici on wheat*

Experiment No.	Intensity: Daily limits. Maximum.	Intensity: Daily limits. Minimum.	Intensity: Total average.	Degree of infection.	Result.	Spore dimensions: Size limits.	Spore dimensions: Modes.
						μ	μ
1	15.0	7.5	10.9	Heavy	20 20	21.28–40.32×16.96–23.68...	32.64×19.84
2	24.7	9.5	14.4	do	16 18	25.60–40.00×16.00–23.04...	33.28×19.84
3	46.5	6.6	14.0	do	18 18	22.40–40.32×16.32–32.68...	32.00×19.84
4	12.8	1.7	6.6	Weak	6 7	22.72–35.84×16.96–22.08...	29.76×19.52
5	10.3	2.0	5.1	do	20 20	21.76–36.48×17.28–22.72	29.12×19.84
6	2.0	0.9	1.4	Moderate	23 23	23.04–40.64×14.72–22.72	29.76×18.88

The rust consistently developed better in fairly high intensities than in the lower ones. The size of the urediniospores, as given in Table XVI, responded in similar manner. The color of the uredinia in the shade varied from antique-brown to Sudan-brown, while of those in the light ranged from Sudan-brown to argus-brown—that is, somewhat lighter in shade than in the open. It appears that in as much as the photosynthetic activities of the host plant are affected by the light intensity in so much does the function and structure of the rust fungus depend on the same factor.

EFFECT OF EXCESSIVE NITROGENOUS FERTILIZATION

The preliminary results obtained by the writers seem to indicate that an excessive amount of sodium nitrate, inhibiting the growth of the host, also inhibits the development of the rust and dimimishes very perceptibly the size of the urediniospores, as is shown in Table XVII. This is in accord with Sheldon's (*10*) carnation rust experiments which showed that the kind of soil that favored the growth of the host also favored the attack of the rust, and that, as a rule, the period of incubation of the rust was inversely proportional to the vigor of the host. The plants were considerably shriveled by the chemical and badly dried two weeks after application. The rust, however, had made a fair start on one blade out of the eight inoculated and developed uredinia of moderate size and extent. The uredinia were darker in color than those developed under normal conditions.

TABLE XVII.—*Effect of excessive nitrogenous fertilization on the physiology and morphology of urediniospores of Puccinia graminis tritici on wheat*

Fertilizer.	Condition of host.	Degree of infection.	Result.	Spore dimensions.	
				Size limits.	Modes.
Sodium nitrate	Shriveled and dried up	Moderate	1/8	28.80–46.80×16.00–23.04	28.48×19.52
Control	Healthy and thrifty	Heavy	20/20	28.28–48.12×16.96–21.68	32.64×19.84

EXPERIMENTS ON CULTURAL METHODS

It was thought well worth while conducting a few experiments to ascertain the effect of the age of the host plant at the time of inoculation on the rust growth and on the size of the urediniospores, also to find out the length of time during which urediniospores retain their vitality and what is the relation of the age of the fungus to the morphology of the urediniospores. The results obtained (Table XVIII) show that the susceptibility of the host is little dependent on its age, and that urediniospores retain their vitality for a considerable length of time with no perceptible variation in size.

TABLE XVIII.—*Results showing the effect of age of host plant on the morphology of urediniospores of Puccinia graminis avenae*

Experiment No.	Host plant.	Age of plants inoculated.	Spore dimensions.	
			Size limits.	Modes.
		Days.	μ	μ
1	*Avena sativa*	7	24.00–35.52×16.00–22.08	29.44×19.20
2	do	7	23.04–35.52×16.96–22.08	29.44×19.52
3	do	14	24.96–34.88×14.72–22.72	29.44×19.20
4	do	21	24.96–35.84×16.00–22.40	29.44×19.20
5	do	21	21.76–37.12×16.64–21.76	29.44×19.20
6	do	35	22.72–35.84×16.00–22.40	29.12×19.20

EFFECT OF THE AGE OF HOST PLANTS ON THE DEVELOPMENT OF THE RUST FUNGUS AND SIZE OF THE URED1NIOSPORES

Oats were here used as host plants, because they were found to thrive better under greenhouse conditions than wheat, barley, or rye. Inoculations were made when the plants were 7, 14, 21, and 35 days old, counting the age from date of sowing. The 7 and 21 days series were later duplicated. In all cases the plants were inoculated with a uniform amount of fresh urediniospore material of *P. graminis avenae* and cultured under similar and normal conditions.

The plants 1 week old were slightly more vigorously affected (Table XVIII) at first, but at the end of 10 days the infection was heavier on the older plants, and especially so on those that were three weeks old at the time of inoculation. From Table XVIII it will be seen that the

size of the urediniospores was remarkably uniform regardless of the age of the host; nor was there any difference in the shape and color of the spores.

The junior author has also obtained very successful infection on mature plants of more than a hundred different varieties of wheat, grown in the greenhouse and artificially inoculated with *P. graminis tritici*. This latter work was conducted at the Kansas Agricultural Experiment Station in cooperation with the United States Department of Agriculture.

EFFECT OF THE AGE OF THE RUST FUNGUS ON THE VITALITY AND MORPHOLOGY OF THE UREDINIOSPORES

Fromme (4, p. 504) states that De Bary found the length of time during which urediniospores of *P. graminis* retain their vitality to vary between one and two months, and that Bolley obtained a 5 per cent germination with urediniospores of *P. graminis* after exposure to air and sunlight during the month of August. The object of this experiment was to determine the vitality of the urediniospores after different periods of association with their respective hosts and effect of the length of association on the rust morphology.

For the determination of the first phase of the experiment inoculations were made (Table XIX) with rust material at different stages of the development of the uredinia. Transfers were made when the uredinia were merely beginning to break through the epidermis and two and four weeks afterwards. There was no apparent difference in the degree of infection produced by these methods of inoculation.

TABLE XIX.—*Results showing the effect of age of the fungus on the morphology of urediniospores of Puccinia graminis*

Experiment No.	Biologic form.	Age of spores measured.	Spore dimensions.	
			Size limits.	Modes.
		Days.	μ	μ
1	*P. graminis tritici*	8	25. 60–39. 68×16. 96–22. 72	32. 64×19. 84
2	do	14	25. 60–40. 32×16. 32–23. 36	32. 96×19. 84
3	do	22	23. 36–39. 04×15. 68–22. 72	30. 72×19. 20
4	do	63	24. 32–40. 96×16. 64–23. 04	32. 64×19. 52
5	*P. graminis avenae*	7	24. 96–35. 52×16. 96–23. 36	29. 44×20. 16
6	do	20	23. 04–35. 20×16. 96–22. 08	29. 12×19. 52
7	do	40	23. 04–35. 52×16. 96–22. 08	29. 44×19. 52
8	*P. graminis secalis*	8	22. 40–34. 24×13. 76–19. 84	28. 48×16. 96
9	do	14	20. 48–33. 92×14. 08–19. 52	27. 52×16. 64

In determining the effect of the age of the fungus on the morphology of the spores, measurements were made at different stages of the development of uredinia, beginning 7 days after inoculation and ending with 63 days. Three biologic forms of *P. graminis* were used in this experi-

ment and, as shown in Table XIX, there seemed to be no appreciable effect on the size of the spores, although the size of the uredinia gradually and persistently became larger, which was due to the additional shedding of spores and coalescence of adjacent uredinia. The color of the uredinia became darker with age and the spores lost their coherent floccose consistency and by the least disturbance were separated from the uredinia.

GENERAL DISCUSSION

The data presented in this paper provide ample evidence to show that the morphology of biologic forms is but slightly and only temporarily changed in response to biotic and physical factors. Resistant host plants and unfavorable cultural conditions affecting the normal development and vigor of the rust fungus may also affect the size of its urediniospores. But as soon as the unfavorable factors are removed the fungus resumes its normal functions and regains its original structure.

No host which is congenial to a given biologic form can, under favorable cultural conditions, exert any perceptible influence on the morphology of the rust spores. *P. graminis avenae* appears to deviate from this rule in so far as shape and size of urediniospores are concerned. The shape varies considerably on any host to which the rust may be confined, while the spore sizes appear to be peculiar of the particular host the rust parasitizes.

The attempts to split up the various biologic forms of *P. graminis* studied into a number of different morphological strains by culturing them for long periods of time on several different but definitely congenial hosts have utterly failed. The attempts to unify the spore sizes of different biologic forms by culturing them continuously for a considerable length of time on the same host were also unsuccessful.

Adverse environmental conditions, such as resistant host varieties, affect the virulence and spore size of the rust fungus. Excessive heat is more injurious to the rust growth and affects the size of urediniospores more effectively than does very low temperature. High humidity during the incubation period appears to be an indispensable condition; the difference in humidity later is probably of lesser importance. Deficiency of soil moisture and sunlight, and other ecological factors affecting the host plant unfavorably, appear to be equally unfavorable to the rust parasite.

The results show that *P. graminis* is quite stable and can not be expected to change rapidly. This is true both of its parasitic capabilities and of its morphologic characters. The facts presented in this paper give additional support to the rapidly accumulating body of data which show that the biologic forms studied are fairly constant. Whether this will apply equally well to the large number of forms recently found on varieties of wheat is a question which can be answered only by future investigation.

SUMMARY

(1) The amount of spore material used for inoculation has no perceptible effect on the result of infection or size of spores, except in so far as a more extensive area of and a greater certainty for successful infection may be secured.

(2) The optimum length of incubation period in the moist chamber is 48 hours, thereby securing all certainty of infection without causing a tendency to supersensibility.

(3) The superficial layer of each uredinium contains larger spores, and when this layer is removed the remaining spores are considerably smaller. But if the uredinium is allowed to produce a new crop of spores those on the surface again attain the same dimensions as the original ones.

(4) In spore measurements 100 spores, obtained from a number of uredinia, are representative of their group. Modes are a practical basis for comparison.

(5) Biologic forms are constant not only parasitically but also morphologically. As a general rule the morphologic differences between the various biologic forms are fully as great and distinct as between many established species of fungi. The morphologic stability of a biologic form is exhibited in the constancy of size, shape, and color of the urediniospores of the particular form. The stemrust of oats (caused by *P. graminis avenae*) is an exception to this rule in so far as the shape and size of urediniospores are concerned, these being very plastic.

(6) Common hosts which are congenial to different biologic forms lack the ability to unify them, as they are unable to exert any influence on the spore morphology. Uncongenial hosts, on the other hand, almost invariably tend to decrease the size of uredinia and spores.

(7) In computing data and comparing results it is necessary to take into consideration the ecological conditions under which the rust had been cultured—that is, cultural conditions should be kept as far as possible uniform; or proper allowances should be made for any variation before final conclusions are drawn.

(8) Adverse environmental conditions unfavorable for the host are also unfavorable for the parasite, affecting the virulence and spore size of the latter.

(9) The optimum atmospheric temperature for the development of the rusts studied appears to range between 66.5° and 70° F. Sufficiency of water and plentiful light are indispensable for the best growth of the rust.

(10) The age of the host seedlings, provided they are healthy at the time of inoculation, has no determining affect on the virulence of infection or size of the urediniospores.

(11) The length of association of a rust with its host, after the first uredinia have burst the epidermis until teliospores are formed, does not impair the viability of the urediniospores, nor does it exhibit any marked and consistent effect on their size.

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